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Marine Ecosystem
Enclosed Experiments

Proceedings of a symposium held
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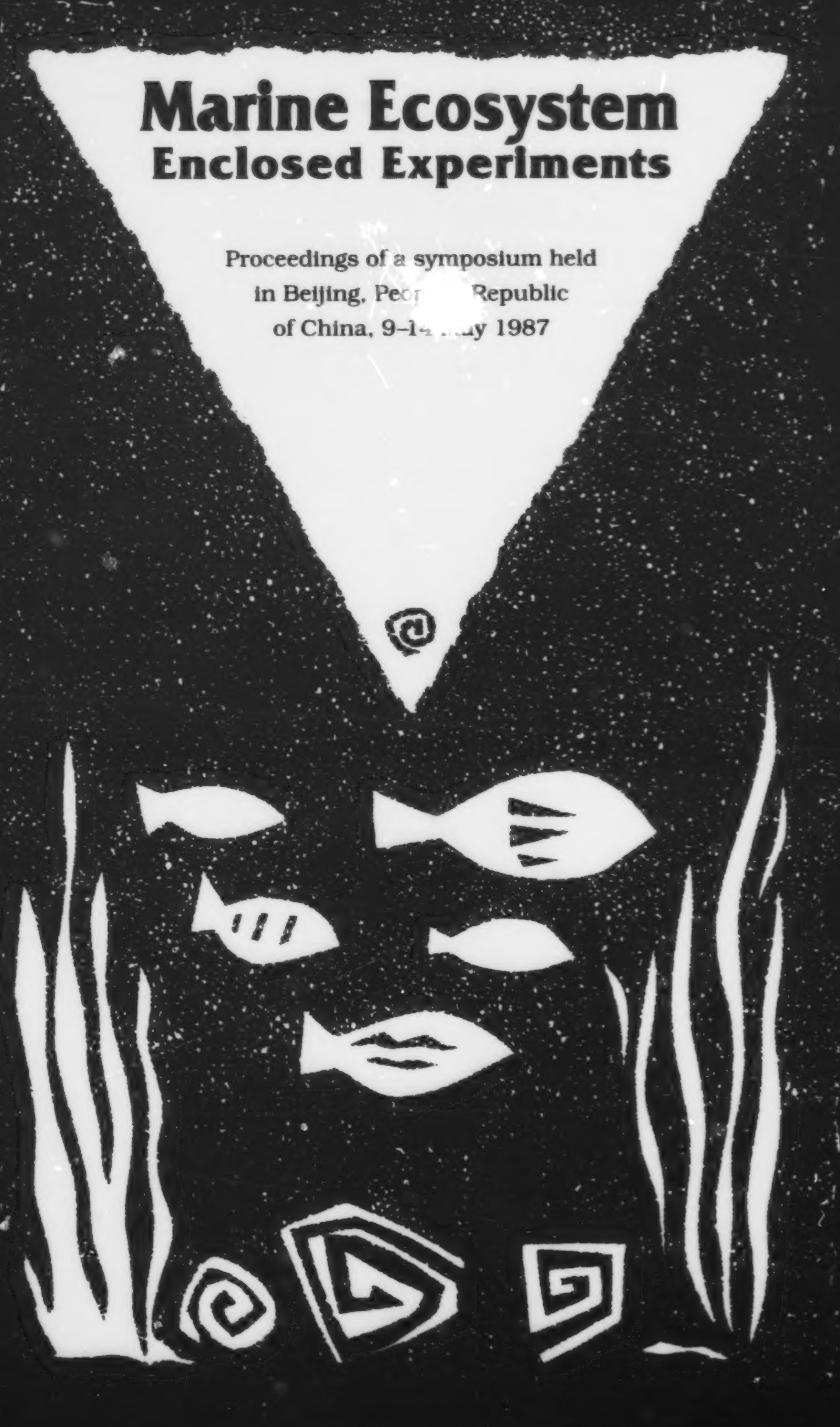

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Marine Ecosystem Enclosed Experiments

Proceedings of a symposium held in Beijing, People's Republic of China, 9–14 May 1987

Editor: C.S. Wong and P.J. Harrison

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Abstract

This symposium on marine ecosystem enclosed experiments (MEEE) consists of nine review papers that describe various types of ecosystem enclosures and a series of papers resulting from enclosure experiments in Xiamen, People's Republic of China, and Saanich Inlet, BC, Canada. The reviews on types of enclosures include benthic enclosures for rocky and sandy shores and the effects of pollutants (primarily hydrocarbons) on bacteria, macroalgae, and invertebrates. The pelagic enclosures were used to study the control of phytoplankton blooms, the uptake and release of dissolved organic substances, and the effects of pesticides on freshwater ecosystems.

Six enclosure experiments were conducted in China and Canada from 1986–87. Some of these experiments examined the effects of contaminated sediments, primarily heavy metals, on bacteria, phytoplankton, and zooplankton and the pathways and fates of these heavy metals in the seawater. Other experiments studied the chemistry and biological effects of chemically dispersed oil.

Résumé

Ce compte rendu du symposium sur les expériences faites en écosystèmes marins comprend neuf communications qui décrivent les écosystèmes retenus et les expériences faites à Xiamen en République populaire de Chine et à Saanich Inlet, C.-B., au Canada. Les communications portent, notamment, sur les écosystèmes benthiques des littoraux rocheux et sablonneux et sur les effets des polluants (surtout les hydrocarbures) sur les bactéries, les grandes algues et les invertébrés. Les expériences sur le contrôle des brutales pullulations ("blooms") du phytoplancton furent menées dans les écosystèmes pélagiques, ainsi que l'absorption et le dégagement des substances organiques dissoutes et les effets des pesticides sur les écosystèmes d'eau douce.

Six expériences ont été faites en Chine et au Canada entre 1983 et 1987. Certaines ont porté sur les effets des sédiments contaminés, principalement par des métaux lourds, sur les bactéries, le phytoplancton et le zooplancton et sur le cheminement et le sort de ces métaux lourds dans l'eau salée. D'autres expériences portaient sur la chimie et les effets biologiques du pétrole dispersé chimiquement.

Resumen

Este simposio sobre Experimentos Marinos en Ecosistemas Cerrados (MEEE) consistió en nueve trabajos de análisis que describen varios tipos de enclaustramientos ecosistémicos y una serie de trabajos derivados de experimentos con estos enclaustramientos en Xiamen, República Popular de China, y en Sannich Inlet, Canadá. Los estudios incluyen enclaustramientos bentónicos para costas rocosas y arenosas, y los efectos de los contaminantes (fundamentalmente hidrocarburos) sobre bacterias, macroalgas e invertebrados. Los enclaustramientos pelágicos se utilizaron para estudiar el control de la reproducción del fitoplancton, la ingestión y expulsión de substancias orgánicas disueltas y los efectos de pesticidas en los ecosistemas de agua dulce.

Se realizaron seis experimentos en ecosistemas cerrados en China y Canadá, de 1983 a 1987. Algunos de estos experimentos examinaron los efectos que ejercen los sedimentos contaminados, fundamentalmente los metales pesados, sobre bacterias, fitoplancton y zooplancton, y el ciclo y destino final de estos metales pesados en el agua de mar. Otros experimentos estudiaron los efectos químicos y biológicos de los aceites crudos dispersados por medios químicos.

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Part I

Introduction

Introduction to the MEEE Project

P.J. Harrison,[1] F.A. Whitney,[2] Wu Jinping,[3] T.R. Parsons,[1] and C.S. Wong[2]

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The use of marine plankton enclosures to study biological processes and marine food chains began three decades ago at Nanaimo, BC, Canada. Strickland and Terhune (1961) used a 6-m diameter spherical enclosure to study chemical and biological changes that occurred during a phytoplankton bloom.

The enclosure concept was revived and modified more than a decade later during an international project called CEPEX (Controlled Ecosystem Pollution Experiment). This project was part of the International Decade of Ocean Exploration, funded by the United States National Science Foundation (NSF). In this project, water columns up to 10 m in diameter and 30 m deep were enclosed in polyethylene enclosures. Experiments were conducted on the effects of various pollutants on the trophodynamics of pelagic ecosystems. This project ran for 6 years at a site on Saanich Inlet, BC, Canada, where the Institute of Ocean Sciences (IOS) is located (Fig. 1).

CEPEX ended in 1979. NSF subsequently funded an international symposium on marine enclosure experiments in 1980, the proceedings of which were published (Grice and Reeve 1982).

The marine enclosure and CEPEX concept were introduced to China by the late Professor Li Faxi of Xiamen University after his visit to Canada in 1979. He submitted a proposal to the Chinese government suggesting that delegates from China attend the CEPEX symposium. Here, Professor Li Guanguo, a visiting delegate from the Peoples's Republic of China, met Professor Parsons from the University of British Columbia and Dr C.S. Wong from IOS. This meeting lead to a cooperative project between China and Canada on marine enclosures. The 5-year (1983–1987) project was named the Marine Ecosystem Enclosed Experiments (MEEE), and was funded by International Development Research Centre (IDRC), Canada, and the State Oceanic Administration (SOA) of China. The Chinese experimental sites were located at the Third Institute of Oceanography, Xiamen, and in Xiamen Bay. SOA financed improvements to the Third Institute of Oceanography's facilities that included constructing a clean room for trace-metal analyses, upgrading

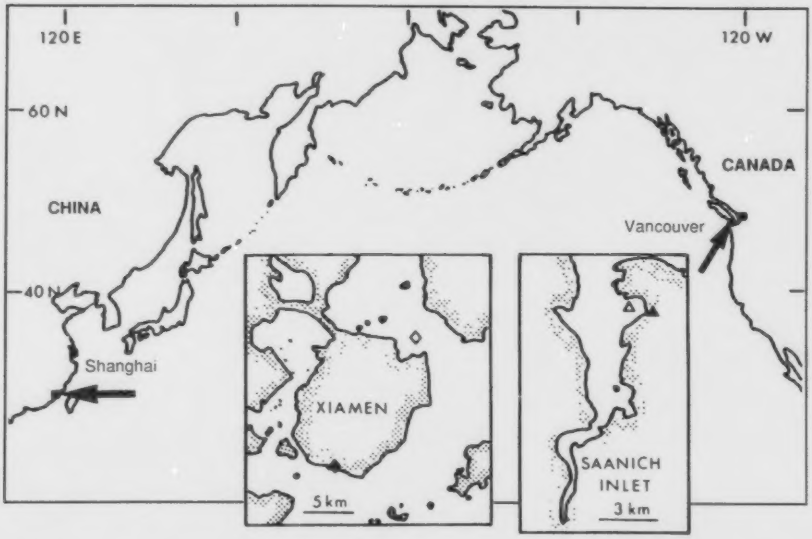

Fig. 1. Enclosure sites in China and Canada bordering the North Pacific Ocean. Insets of Xiamen show the Third Institute of Oceanography (closed diamond) and MEEE catamaran (open diamond) and of Saanich Inlet show the Institute of Ocean Sciences (closed triangle) and the controlled experimental ecosystem floats (open triangle).

seawater ponds, and manufacturing a 15-m launch and a catamaran. IDRC contributed major funding for laboratory and field equipment, travel, and living expenses.

Among the many people who supported the MEEE program throughout its existence were Rachel Des Rosiers (IDRC), Luo Yiru (former Director-General of SOA), Chen Guozhen (Chairman, Science and Technology, SOA), Cao Pifu (Head, Foreign Affairs, SOA), Wong Jianwen (Director, Science and Technology, SOA), and Chen Bingxin and Xu Canglong (former directors of the Third Institute of Oceanography).

The main objective of the MEEE project was to conduct a joint research program between Canada and China to strengthen marine pollution research in China. The specific aims of the project were five-fold:

- To understand the pathways and fate of toxic chemicals in the marine environment by conducting experiments on enclosed marine ecosystems;
- To assess the effects, both chemical and biological, of toxic chemicals using the MEEE technique;
- To establish levels of chemicals in ocean waters, in particular ultratrace metals;
- To train scientists in the techniques necessary (i.e., MEEE approach, clean-room methods, etc.) to acquire such data; and
- To hold a symposium on the results of the project to determine how best to

use the knowledge obtained to promote marine environmental protection in China.

Participants in the project came from IOS (Department of Fisheries and Oceans, Canada); the Department of Oceanography at the University of British Columbia; the Third Institute of Oceanography, Xiamen; and the Ocean University of Qingdao (formerly Shandong College of Oceanography). Scientists from Xiamen University, the Institute of Marine Environmental Protection of SOA, and other Chinese institutes also joined in the experiments both in China and in Canada.

Phase I of MEEE began with a trip to China to meet the Chinese scientists that would be involved in the project, to formulate the experiments, and to assess what equipment and supplies would be required to conduct the experiments. An initial MEEE experiment on the dispersion of crude oil took place in 1983 at IOS under Phase I funding.

Phase II involved two experiments in China and three in Canada; a 6-week training course at the University of British Columbia for 10 scientists; two fellowships for Chinese master's degree graduate students; a series of lectures by scientists who were visiting China; an international symposium on marine enclosures held in Beijing, 9–14 May 1987; and publication of the symposium proceedings. Participating in each of the experiments in Canada were four or five Chinese scientists who came to IOS for periods of 3–4 months for on-site training. During experiments conducted in China, six to eight Canadian scientists stayed in Xiamen for 4–8 weeks to assist in setting up and conducting experiments. In all, over 50 Chinese and 15 Canadian scientists took part in these enclosure experiments.

MEEE experiments

1983

Three polyethylene-walled controlled experimental ecosystems (CEEs, 2.5-m diameter × 16-m deep) (Menzel and Case 1977) were deployed in Saanich Inlet, BC, at the former CEPEX site (near 48° 40′ N, 123° 28′ W) in water 20 m deep. Between 17 July and 25 August, the fate and effects of chemically dispersed Prudhoe Bay crude oil were studied (Parsons et al. 1984; Whitney 1984; Wong et al. 1984; Lee et al. 1985; Harrison et al. 1986).

1984

Over 17 days (7–24 August), three CEEs were used at the IOS site to observe the impact of mine tailings and heavy metals leached from the tailings on a marine ecosystem (Parsons et al. 1986; Wong et al. 1986).

1985

At the Third Institute of Oceanography, Xiamen, a land-based granite tank (20 m × 10 m × 5 m deep) was repaired and filled with seawater pumped from Xiamen Bay. Nine polyethylene bags (2 m diameter × 4 m deep) were mounted on floating wooden modules and filled simultaneously with water taken from a depth of 3 m at a point 150 m offshore where the water was 12 m deep (Wu J., et al., this volume). Between 18 April and 15 May, seven of these ecosystems were in contact with either Xiamen Harbour sediments or low to moderate levels of trace metals.

From 12 August to 6 September, five fibreglass portable marine enclosures (PMEs, 1 m diameter × 2 m deep) supported by a catamaran (Wong et al., this volume), moored at the IOS boat wharf, were sampled. Metal fluxes from sediment beds of False Creek dredgeates (Vancouver, BC, harbour) were measured in environments that were either dark, oxygenated, anoxic, or turbid (silty).

1986

A catamaran that supports four marine ecosystem enclosures (MEEs, 2 m diameter × 6 m deep) was anchored in Xiamen Bay (24° 31′ 30″ N, 118° 5′ 25″ E) about 20 km north of the Third Institute of Oceanography. From 23 April to 5 June, the effects and fate of Shengli crude oil were monitored. Further details of the Chinese sites and enclosures are given by Wu J. et al. (this volume).

The final MEEE experiment was held at IOS, 3–24 October. The fates of Shengli crude oil and low concentrations of trace metal were followed in four darkened PMEs.

Results from 1985 and 1986 experiments are included in this publication.

Grice, G.D., Reeve, M.R., ed. 1982. Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 430 pp.

Harrison, P.J., Cochlan, W.P., Acreman, J.C., Parsons, T.R., Thompson, P.A., Dovey, H.M., Chen, X. 1986. The effects of crude oil and Corexit 9527 on marine phytoplankton in an experimental enclosure. Marine Environmental Research, 18, 93–109.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parsons, T.R., Lalli, C.M., Wu, J. 1985. Microbial response to crude oil and Corexit 9527: SEAFLUXES enclosure study. Microbial Ecology, 11, 337–351.

Menzel, D.W., Case, J. 1977. Concept and design: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 1–6.

Parsons, T.R., Harrison, P.J., Acreman, J.C., Dovey, H.M., Thompson, P.A., Lalli, C.M., Lee, K., Li, G., Chen, X. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527: part 2 — biological effects. Marine Environmental Research, 13, 265–275.

Parsons, T.R., Thompson, P., Wu, Y., Lalli, C.M., Hou, S., Xu, H. 1986. The effect of mine tailings on the production of plankton. Acta Oceanologica Sinica, 5, 417–423.

Strickland, J.D.H., Terhune, L.D.B. 1961. The study of in situ marine photosynthesis using a large plastic bag. Limnology and Oceanography, 6, 93–96.

Whitney, F.A., ed. 1984. The effects and fate of chemically dispersed crude oil in a marine ecosystem enclosure — data report and methods. Institute of Ocean Sciences, Department of Fisheries and Oceans, Sidney, BC, Canada. Canada Data Report of Hydrography and Ocean Sciences 29, 77 pp.

Wong, C.S., Whitney, F.A., Cretney, W.J., Lee, K., McLaughlin, F., Wu, J., Fu, T., Zhuang, D. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527: part 1 — fate of chemically dispersed crude oil. Marine Environmental Research, 13, 247–263.

Wong, C.S., Whitney, F.A., Johnson, W.K., Lu, X., Wu, J., Wu, S., Li, and J. 1986. The effect of mine tailings on levels of heavy metals in seawater. Acta Oceanologica Sinica, 5, 401–415.

Summary

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This symposium was the third international symposium on marine mesocosms. The first symposium was held in 1977 in Edinburgh, Scotland, as part of the Joint Oceanographic Assembly, and focused mainly of mesocosm design (Parsons 1978). The second was held in 1980 at the Institute of Ocean Sciences, Sidney, BC, Canada, and dealt exclusively with plankton mesocosms (Grice and Reeve 1982). In 1985, a Scientific Committee on Ocean Research (SCOR) working group was set up to review applications of mesocosms to ocean research (Lalli 1990) and prepare a manual of Marine Experimental Ecosystems (Rokeby 1991). The third symposium was held in Beijing, People's Republic of China, the papers of which make up this volume and cover both planktonic and benthic mesocosms. Papers on benthic studies reviewed the intertidal mesocosms used by European researchers.

There were three invited speakers and 37 contributed papers for this symposium. The first invited speaker, Dr M.E. Pilson, reviewed the Marine Ecosystems Research Laboratory (MERL) mesocosm facility at the University of Rhode Island. This is a land-based facility that has been used to examine responses of plankton and benthos to pollutants and environmental factors. The system is often used for long term continuous-flow experiments to simulate Narragansett Bay. Experience with MERL mesocosms has shown that many aspects of Narragansett Bay ecosystem behaviour appear to be effectively captured, especially if the flow-through mode is used. This is most clearly shown in various examples of biogeochemical processes.

Dr K.R. Solomon from the Canadian Centre for Toxicology reviewed the use of freshwater mesocosms or limnocorrals to study the effect of pesticides on lake ecosystems. These freshwater mesocosms in small lakes can be low in cost compared with marine mesocosms because of reduced wave action and tidal currents, etc. Replication allowed for statistical analyses of the data and pesticide dose–response effects with respect to both the degree of the effect and the response time on a variety of planktonic species were obtained.

Dr H. Farke described the Bremerhaven Caissons, which are used to study the effects of pollutants such as oil on intertidal sand flats and mud flats. The caissons are actually large boxes that are open at the top and bottom and enclose an area of about 13 m^2. They can be operated in a closed, semiclosed, or flow-through mode. Oil pollution experiments were run in the semienclosed mode, where the water inside the caisson is exchanged with each tidal cycle. Because refloating at the end of the experiment is possible without severely disturbing the site, postexperimental effects can be investigated under natural environmental conditions.

The contributed papers can be divided into three groups: mesocosm techniques, natural ecosystems, and impacted ecosystems. A variety of different designs for plankton mesocosms used in harbours, fjords, and the open sea was reviewed by Dr U.H. Brockman, Germany. The enclosures were made of heavy, transparent plastic, and were 1 m in diameter and varied in length from 3 to 40 m. They have been used to study fluxes of natural compounds (e.g., the release of dissolved organics), phytoplankton succession, the development of copepod cohorts and bacteria, and phytoplankton and zooplankton interactions. Drifting mesocosms in the open sea have been used to investigate ecological processes at extremely low nutrient concentrations.

Enclosures developed since the Controlled Ecosystem Pollution Experiment (CEPEX) to study the pathways and fate of chemical pollutants were described by F. Whitney. Enclosure techniques recently employed by the Institute of Ocean Sciences, Canada, included: first, benthic boundary enclosures with the container attached to the bottom sediment; second, suspended enclosures with a sediment pan at the bottom; third, fibreglass tanks suspended from a barge; and, fourth, a mesocosm suspended from an easily assembled float for in-situ studies. These enclosures were used to study the effects of metals on plankton, metal release from contaminated sediments, and effects of mine tailings, oil, pentachlorophenol, and polychlorinated biphenyls (PCBs) on marine ecosystems.

Enclosure techniques for intertidal mud flats were presented by Dr P.A.W.J. de Wilde, Netherlands. Eight Model Tidal Flats (MOTIFs) have been built outdoors to study the environmental effect of large and small artificial oil spills, the use of dispersants, and other oil-combating techniques. Sediment and associated benthic organisms are brought to the tanks near the laboratory and oil-effect studies are initiated. Both acute effects (deviant behaviour and increased mortality in macrofauna, meiofauna, and zooplankton; enhanced primary productivity; and bacterial activity) and long-term effects (retarded larval settlement and ecosystem recovery) could be observed. Bioturbation by lugworms was found to be a important process in trapping oil in the sediment. In addition to indoor mesocosms, two subtidal North Sea mesocosms are now under construction.

Sediment and associated benthic organisms from a Norwegian fjord were transplanted into two large indoor basins and studied over a 6-month period by Dr J.S. Gray. Communities established in the mesocosm closely simulated the natural community. Mobile species, such as amphipods, were underrepresented in the mesocosm and recruitment of larvae was reduced. Experiments with this system include simulating sedimentation of the spring diatom bloom and following the response of bacteria, meiofauna, and macrofauna. Photography proved to be very useful to keep track of individual worm activities.

Large, littoral, hard-bottom community mesocosms have been established for oil-pollution experiments in Norway by Dr T. Bakke. The communities are enclosed in concrete basins equipped with artificial wave and tidal simulation, and a water-exchange system to ensure a supply of recruitment stages of algae and animals. The rocky-shore mesocosms can be operated for several years, enabling the investigator to undertake studies on the effects of chronic perturbations of plant and animal populations. One problem associated with interpreting results from long-term experiments was natural deviation among communities with time because of slight initial differences in community structure and subsequent recruitment.

Two papers dealt with natural ecosystems. Mesocosm experiments conducted in coastal waters of Xiamen, People's Republic of China, indicate that phosphate is the nutrient that limits phytoplankton production. During these mesocosm experiments, Dr P.J. Harrison and colleagues found that phosphate always reached undetectable levels several days before inorganic nitrogen. This observation was supported by the N:P ratio in the water at the beginning of the experiments, which was nearly 80:1. This represents a fivefold excess of nitrogen, assuming that phytoplankton take up nitrogen and phosphorus in a ratio of 16:1. These coastal waters are unique in that marine waters are usually nitrogen-limited. The consequences of phosphorus limitation are that phosphorus-limited phytoplankton may be more sensitive to pollutants than nitrogen-limited cells.

The natural ecosystem off the west coast of Canada has been studied in the Subarctic Pacific Ecosystem Research program (SUPER) by Dr M.R. Landry and colleagues. The goal of the program is to understand the biological, chemical, and physical mechanisms that prevent blooms of phytoplankton from occurring in the oceanic subarctic Pacific. Sixty-litre microcosms were used on board ship to study phytoplankton growth with and without copepod grazers and with and without added ammonium. These shipboard microcosms, which were continuously rotated in a seawater-cooled bath at about the mean light level of the upper mixed layer, provide an excellent shipboard facility to manipulate physical, chemical, or biological factors and to follow the dynamics of phytoplankton and zooplankton responses. This microcosm approach is a more reliable way of alleviating advection effects than tracking a water mass with a drogue.

The remainder of the contributed papers examined the impact of pollutants, such as oil or heavy metals, on ecosystems primarily off the coasts of China or Canada. Mr J. Wu presented an overview of the mesocosm design and experimental results of two experiments that were conducted at Xiamen, Peoples's Republic of China. The first experiment studied the effect of suspended sediments taken from the Xinglin chemical fertilizer factory in Xiamen and a mixture of heavy metals (Cd, Cu, Hg, Pb, and Zn) on plankton communities from Xiamen Bay. Mr M.H. Hou reported on phytoplankton species succession, whereas Mr X. Chen showed the effects of these heavy metals on primary productivity. Dr C.M. Lalli compared the response of herbivorous copepods and larvaceans in experiments conducted in China and Canada. In Canada, the addition of mine tailings sediment to the water column caused an increase in the number of copepods and larvaceans, possibly by both delaying and increasing primary productivity. In China, the addition of polluted sediment decreased the number of copepods. It is interesting to note that zooplankton species were remarkably similar considering that temperate (Canada) and subtropical (China) environments were being compared.

The pathway and fate of the heavy metal mixture used in the Xiamen experiments was summarized by Mr K. Xu and Mrs J. Li. Scavenging by settling particles was the major removal mechanism of dissolved Hg, Pb, and Zn, whereas Cd and Cu remained in the dissolved form. Most heavy metals were weakly associated with zooplankton. Mr H. Zou and co-workers reported on a specific experiment in which the fate of ^{65}Zn was followed. During the first part of the experiment, Zn remained dissolved, but later it adsorbed onto particles and was removed from the water column when these particles sank. The release of heavy metals from Xiamen Bay polluted sediment was reported by Mr G. Zhang and colleagues. Dissolved Co, Fe, and Pb reached their maxima during the first 24 h and then decreased to near background values, whereas Cd and Cu showed an initial increase after addition of the heavy metal mixture and remained at elevated concentrations throughout the experiment. Zinc and nickel did not show elevated concentrations.

The concentration of metals in seawater outside the enclosures (i.e., Xiamen Bay seawater) was also measured by Mrs J. Li and colleagues. They found their concentrations to be much lower than previously reported values because they were able to minimize contamination by using the new clean-room facility at the Third Institute of Oceanography, Xiamen. Distribution coefficients (K_d) of metals between particles and water follow the order Fe > Co > Pb > Zn > Ni > Cu > Cd. This order shows that Cd and Cu exist primarily in the dissolved form in contrast to Fe, which occurs primarily as particulate Fe. Biogeochemical transformation of Hg was studied by Mr K. Xu and colleagues. They found that a significant amount of inorganic Hg was transferred into the particulate organic form during the phytoplankton bloom. After the bloom, dissolved inorganic Hg increased again, implying that Hg that had been taken up by the phytoplankton was released during decomposition of the bloom.

Several metal- or sediment-release experiments were conducted in Canada. Enclosure experiments were conducted by Dr C.S. Wong and colleagues to investigate the fluxes of Cd and Pb from dredged sediments and to determine if metal release from the sediments could be prevented by capping the sediments with alluvial materials. The capping material did not prevent Cd from being released from the sediments. In fact, Cd was released from the capping material itself. In contrast, the capping material absorbed Pb released from the sediments and reduced by 50% the amount of Pb being released into the water column compared with that released into the water column from the uncapped sediment.

The release of metals (Cd, Cu, Fe, Pb, Ni, and Zn) from mine tailings obtained from Alice Arm, BC, Canada, was reported by Mr B. Zhan. Primary productivity and chlorophyll concentrations were severely reduced as a result of adding 100 ppm of mine tailings. Iron and zinc decreased after the mine tailings were added, whereas Cd, Cu, Ni, and Pb increased, suggesting that these metals were released from the mine tailings.

Flux, speciation, and a Hg budget were studied in enclosures in Saanich Inlet, BC, Canada, by Mr X. Lu and colleagues. Removal of Hg in the water column was described by first-order kinetics for both total and particulate Hg. The removal rate depended on the magnitude of primary production and species composition of the phytoplankton. The half-life for total Hg varied from 2.8 d for a diatom bloom to 30 d for a microflagellate bloom.

Twelve contributed papers dealt with oil pollution. Two papers reviewed highlights from a number of experiments on oil degradation. General conclusions were drawn from these experiments by Dr W.J. Cretney. Addition of oil causes a rapid increase in bacterial numbers as biodegradation begins. The low-volatility n-alkane fraction of the oil is degraded first. Bacteria and oil particles (from which volatile and soluble hydrocarbons have been leached) become associated in a floc that remains in suspension. These flocs can be rapidly sedimented by association with sedimenting phytoplankton. Dr K. Lee discussed how chemical dispersion, microbial seeding, nutrient enrichment, and trophic-level interactions affected rates of microbial degradation.

Seven papers reported on the biological effects of oil pollution. The effects of Chinese Shengli crude oil and the dispersant Corexit 9527 on phytoplankton were studied (Mr X. Chen and colleagues). Diatoms were very sensitive to the oil, whereas pennate diatoms, represented by *Nitzschia closterium*, were more resistant. A similar result was obtained in experiments conducted in Canada (Mr L. Yu and colleagues). The oil used in these experiments also caused a decrease in zooplankton biomass (Mr X. Chen). The dispersant alone had no effect on phytoplankton or zooplankton. The dispersed crude oil stimulated bacterial production (Mr R. Lin and colleagues and Mr Y. Lin and colleagues). Bacterial degradation appeared to be the reason for the marked decrease in short-chained alkanes.

The effects of Chinese Bohai (Mr L. Shen and colleagues) and Liaohe (Mr L. Zhu and colleagues) crude oil were tested on phytoplankton from the coastal waters of the Huanghai Sea (northern China). Both types of oil caused a shift in phytoplankton species composition from large diatoms to microflagellates or smaller diatoms. The dispersant Shuangxiang No. 1 by itself was inhibitory to phytoplankton.

Three papers dealt with the fate of Chinese Shengli crude oil in enclosures. Nonvolatile n-alkanes quickly adsorbed onto suspended particles (Mr S. Wu and colleagues and Mr D. Zhuang and colleagues). Short-chained alkanes (C11–C20) were degraded by microorganisms and longer chained alkanes (C15–C32) settled to the sediment. About 40% of the n-alkanes that were added were recovered from the sediment and bag wall, whereas about 60% were decomposed by microorganisms. A more precise account of the fate of the dispersed crude oil was obtained using the tracer n-(1-^{14}C) hexadecane (Mr W. Li and colleagues).

Three papers reported on the relationship between particulate organic matter formation and biological processes (Mr L. Guo and colleagues, Mr T. Fu and colleagues, and Mrs Z. Xia and colleagues). Particulate organic carbon, nitrogen, and phosphorus were measured during the enclosure experiments and were correlated with chlorophyll production and primary productivity.

A synthesis of the experiments was attempted in three modeling papers. The effects of vertical mixing on ecosystem dynamics in an enclosure were examined using a computer-simulation model (Dr T.R. Parsons). Over a range of turbulent diffusivity from 0 to 1 $cm^2 \cdot s^{-1}$, changes in the standing stock of plankton were followed for a simulated period of 60 d. The effect of increased turbulent diffusion was to cause better phasing between primary, secondary, and tertiary producers, resulting in a higher standing stock of tertiary producers. Conversely, the highest standing stock of phytoplankton occurred in simulations where vertical diffusivity was zero. The reason for this response is that the phytoplankton bloom and sink before the zooplankton are developed and capable of grazing the phytoplankton.

A second numerical model, constructed by Dr T. Kessler, was used to probe the effect of deviations from steady-state conditions on the particulate carbon flux. An ecosystem experiment that assessed the effect adding suspended sediment showed a suppressed phytoplankton growth rate and enhanced zooplankton biomass. Kessler's model reveals the potential importance of the finite-grazing response by zooplankton to a time-dependent algal growth rate.

In the third model, Mr J. Zeng developed a model in which the recovery of phytoplankton from a pollutant was emphasized.

The symposium concluded with an open discussion on the use of mesocosms as a research tool to study ecosystem dynamics. It was concluded that mesocosms are useful to help simplify the ecosystem by eliminating advection problems, thereby allowing the investigator to sample the same biological communities to obtain a time-series response. Several participants stressed the importance of also understanding the natural ecosystem. This can be accomplished by emphasizing the results of the control bag, sampling outside the enclosures, or both. Therefore, good background (baseline) information is essential before biological responses to pollutants in enclosures can be properly interpreted.

A lively discussion on replication and statistical analysis of enclosure data yielded several general conclusions. First, if mesocosms are very large (e.g., CEPEX with 2 000-t enclosures), the cost of replication to study three trophic levels (e.g., phytoplankton, zooplankton, and fish) may be so high that it would not be warranted. Second, the many experiments conducted by MERL reveal that chemical parameters replicate very well (e.g., ±5%). However, biological parameters are highly variable and, depending on the hypothesis being tested and the trophic level involved, replication may be advisable. This replication may be conducted in microcosms (1–10 L) for phytoplankton or zooplankton responses.

The general conclusion was that mesocosms should not be used alone. They should be used in concert with field studies (to obtain baseline data on the natural ecosystem) and microcosms (to assist with replication, testing specific hypotheses for microplankton, or designing future mesocosm experiments by testing dose–response relationships). Attention should be paid to identifying key biological processes that should be measured, developing appropriate time and spatial scales over which to measure these processes, designing the mesocosm, and being aware of artifacts that can be caused by enclosure-wall effects.

Grice, G.D., Reeve, M.R., ed. 1982. Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 430 pp.

Lalli, C.M., ed. 1990. Enclosed experimental marine ecosystems: a review and recommendations — A contribution of SCOR WG 85. Springer-Verlag, New York, NY, USA. Coastal and Estuarine Studies 37, 218 pp.

Parsons, T.R. 1978. Controlled ecosystem experiments: introduction. Rapports et Proces-Verbaux des Reunions, Conseil International pour l'Exploration de la Mer, 173, 5–6.

Rokeby, B.E., ed. 1991. Manual on marine experimental ecosystems. Scientific Committee on Ocean Research, UN Educational, Scientific and Cultural Organisation, Paris, France. 178 pp.

Part II

Applications of Marine and Freshwater Enclosures

Use of Rocky-Shore Mesocosms in Pollution Research[1]

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Four 25-m^3 experimental, rocky-shore communities have been established at the Marine Research Station Solbergstrand, Norway. The communities are enclosed in concrete basins equipped with artificial wave and tide simulation and a water-exchange system to ensure an adequate supply of recruitment stages of algae and animals. The communities were established by transplantation and natural recruitment between 1979 and 1982. They have recently been used in a 3-year experiment on the effects of continuous exposure to low concentrations of diesel-oil hydrocarbons. The experiment has shown that rocky-shore mesocosms can be run successfully for several years, enabling complex studies of the effects of chronic perturbations, especially with respect to populations and individuals, to be carried out. The size of the model community is critical, particularly in relation to sampling requirements, but in hard-bottom communities, this can be minimized through the use of nondestructive sampling. The wave and tide system should have a built-in stochastic element because communities that have developed under the physical constancy of the present system are vulnerable to even slight maladjustment of, for example, the water level. A major problem encountered in interpreting results has been natural deviation among the communities over the years because of slight differences in community structure and recruitment. Deviation may, to some extent, be counteracted by manipulating the population sizes, but strict manipulation should be avoided because it represents an unnatural forcing factor on the system. Long-term benthic mesocosms must be regarded as a series of fairly similar and partly independent communities, not as experimental replicates in laboratory terms.

In the context of experimental model ecosystems, little attention has been paid to the hard-bottom benthos. Most mesocosm systems deal with the pelagic environment, subtidal soft bottoms, salt marshes, or other soft shorelines. The most comprehensive hard-bottom systems reported in the literature are the estuarine *Fucus vesiculosus* community mesocosms at Karlskrona in Sweden (Notini et al. 1977) and the marine rocky-shore mesocosms at the Marine Research Station Solbergstrand in eastern Norway (Bakke 1986; Gray 1986).

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[1] Marine Research Station Solbergstrand, Contribution 23.

The paucity of hard-substrate mesocosms available is, in a way, surprising as hard-bottom communities in both littoral and sublittoral zones should lend themselves to mesocosm experimentation. The physical structure is, in essence, two-dimensional. Artificial substrates can be created using concrete or rocks, or both, and, because most organisms are sessile, they can be moved into mesocosms without much disturbance by transplanting their substrate rocks. One of the reasons why only a few rocky-substrate mesocosms have been established could be that in-situ experiments can easily be performed in hard-bottom communities, especially in the littoral zone (e.g., Dayton 1975; Lein 1980; Bonsdorff and Nelson 1981). However, there are several situations where in situ experiments are not practical for logistic or other reasons and where mesocosms are the best alternative.

The purpose of this paper is to report on certain advantages and disadvantages of using the rock-littoral mesocosms established at the Marine Research Station Solbergstrand, and to assess this approach in comparison with in-situ experiments.

Rocky-shore mesocosm design

The Solbergstrand mesocosms were established in 1979. They consist of four outdoor concrete basins, each 8 m × 5 m × 1.5 m and containing 25 m^3 of seawater at midtide (Fig. 1). A wave generator, consisting of two heavy-duty polyvinyl chloride (PVC) pipes in a steel frame, runs along the entire side of each basin (Fig. 2). The wave generator moves up and down mechanically (18 $strokes \cdot min^{-1}$), creating regular waves running across the basin to the other side where the main model community is established on a series of steps constructed to imitate a slanted shore.

The basins are supplied with running seawater pumped by impeller pumps from

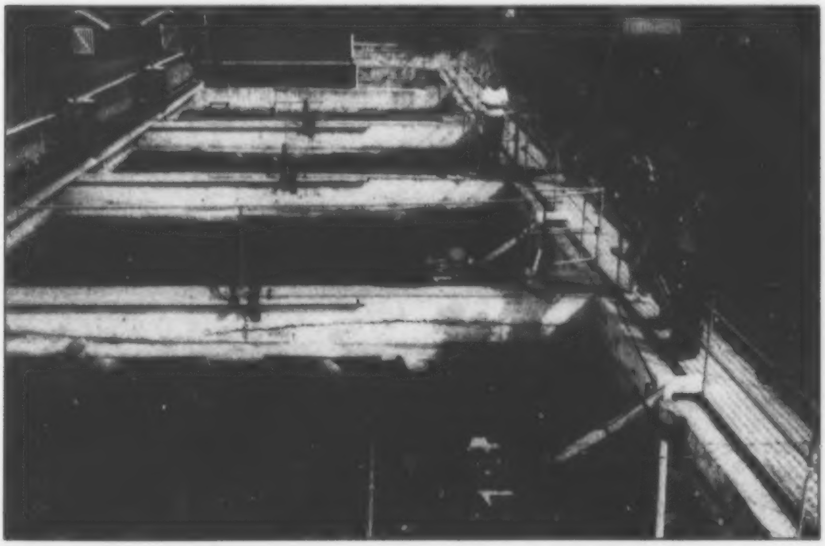

Fig. 1. Four concrete basins used to establish Solbergstrand mesocosms.

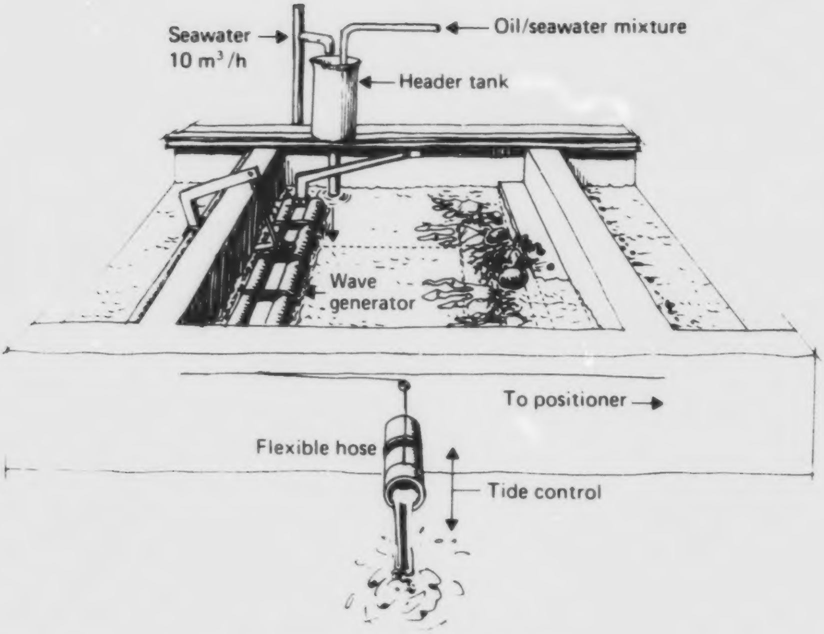

Fig. 2. Schematic of one of the basins.

a depth of 1 m in the fjord outside. The water enters the basins through a header tank, ensuring a stable flow rate of 10 m^3 per basin. The water exchange rate is normally in the range of 2–4 h. From each basin, a subsurface outlet leads to a flexible hose with a stiff nozzle that is raised and lowered automatically to regulate the basin water level in a 12-h sinusoidal cycle following the tidal rhythm and nominal amplitude (30 cm) on the shore. During extreme winter temperatures, water may be taken at 13 m depth to prevent ice formation in the basins.

The first community was established in October 1979. Rocks with algae and animals were transplanted from the littoral zone to the basins and positioned on the steps at a proper tidal level. The basins were then run without any manipulation for 3 years to let the communities develop and stabilize through self-propagation and through larvae and spores entering the basins with the water. At the end of this period, the basin communities contained about 50 species of macroscopic algae and animals. The community structure was typical for medium-sheltered shores of the middle and outer Oslofjord region.

Oil experiment

The mesocosms were established to perform long-term experiments on the effects of water-transported pollutants. Recently, the communities have been used in an experiment on the effects of chronic oil discharge. For 2 years, starting in September 1982, two of the communities were continuously exposed to a wateraccommodated fraction (WAF) of diesel oil in seawater, while the other two were run as controls. The WAF was produced by continuously mixing seawater and oil at a ratio of 1 800:1 in a 580-L mixing chamber. After removing excess oil through automatic surface skimming, the WAF was pumped into the header tank of the exposed basins at nominal dilutions of 75:1 and 300:1, giving average oil concentrations of 129.4 ± 33.3 (SD) and 30.1 ± 10.3 $\mu g \cdot L^{-1}$ in the water of the two basins. After the 2-year exposure period, the basins were observed for another year to study their recovery from the effects of the oil.

Overview of results

The studies performed during the exposure and recovery periods looked at the following:

- Hydrocarbon concentration in basin water, in primary growth on rock, and in tissues of *Ascophyllum nodosum, Fucus serratus, Mytilus edulis*, and *Littorina littorea*;
- Gross community structure and population size of macroscopic algae and animals;
- Colonization pattern, growth, metabolism, and the effects of grazing on primary rock communities;
- Individual growth of *A. nodosum, Laminaria digitata, Ulva lactuca*, and *L. littorea*;
- Population dynamics and genetics of *Balanus balanoides, L. littorea*, and *M. edulis*; and
- Energy conversion and utilization, and biochemical, cytochemical, and histological stress responses in *M. edulis* and *L. littorea.*

These subprojects were performed by individual researchers and students and are, at present, being published in various journals. Summaries of the results are given by Gray (1987) and Bakke (1986). Some of the main effects recorded include the following: a population collapse in *M. edulis* (Bokn and Moy 1985) and amphipods (Bakke, unpublished data); reduced growth in *M. edulis* (Thome and Walday 1984), *A. nodosum*, and *L. digitata* (Bokn 1985); reduced recruitment in *L. littorea* (Lystad and Moe 1985); reduced photosynthesis in the primary rock community (Pedersen 1985); increased cover of opportunistic green algae; reduced feeding and energy utilization in *M. edulis* (Widdows et al. 1985) and *L. littorea* (Bakke 1985); and clear signs of biochemical and cytological stress in *M. edulis* and *L. littorea* (Livingstone et al. 1985; Lowe and Pipe 1985; Moore et al. 1985).

Both algae and animals accumulated oil hydrocarbons in their tissues (Sporstol and Oreld 1985). The population genetics of *M. edulis* did not demonstrate any short-term selection due to the oil (Fevolden and Garner 1986). The severity of effects, in most cases, was dependent on the season, but was not always related to dosage. After the 1-year recovery period, most responses returned to normal.

How well do the mesocosms replicate nature?

A model ecosystem will never copy nature in all respects, but the better a mesocosm imitates natural conditions, the stronger the predictive value of any experimental conclusions. Certain requirements, regarding physical conditions and biological interactions, must be fulfilled in a good mesocosm design.

Physical condition

In a model ecosystem, the physical environmental conditions, with respect to substrate area and character, and water regime should be as natural as possible for the organisms and populations under investigation. This is a prerequisite if the organisms are to respond to a perturbation as they would in nature. The physical environmental constraint of the mesocosm should not in itself be a forcing factor interfering with experimental manipulation.

The model must be large enough to provide the populations under investigation necessary moving space. In rocky-shore mesocosms, this condition is easy to achieve as most of the organisms are sessile or show little migratory movement. Migratory fish demanding larger space were not part of the model community. The total hard-substrate area in each basin is 70 m^2, and the area of the main community space, the steps, is 20 m^2. The basin size is comparable to numerous smaller land-locked bays along the Norwegian coast and should, therefore, represent an adequate realm for the motile fauna of the community, mainly periwinkles, shore crabs, amphipods, isopods, and starfish. A disadvantage of the basins is that the shallow depth prevents motile animals from seeking deep-water shelter as they would normally do during periods of extreme temperatures, but this was counteracted by use of deep water during winter.

The substrate of the basins is a mixture of granite rock and concrete. Except for some of the macroalgae (e.g., *A. nodosum*), which are only found on the rocks on which they were transplanted, there was no obvious difference in the community pattern on neighbouring rock and concrete surfaces. Cracks in the concrete and crevices between the rocks represent shelter from predation for small and juvenile animals.

Hydrographic regime

The high water turnover ensured similar temperature and salinity in the basins as on the shore most of the time. Long-term seasonal change in the temperature followed the surface of the fjord closely, but the basins heated up slightly faster during spring and cooled slightly faster during autumn than the fjord. The mean monthly temperature for the period 1980–1985 ranged from 0.6°C in March to 18.9°C in August compared with 0.4°C and 18.3°C, respectively, in the fjord (Bakke 1986). Similarly, the mean salinity ranged from 30.5‰ in January to 17.8‰ in June in both the basins and the fjord. During extreme summer heat, the basin-water temperature exceeded that of the shore water by a maximum of 2.6°C over the years 1980–1986, but no mortality due to this raised temperature has been reported for any of the populations under investigation. Elevated surface temperature during the summer is also a common natural phenomenon in small shallow bays in the Oslofjord.

Switching to a water supply from 13 m depth during the winter not only prevented ice formation in the basins but also produced water conditions similar to those that the motile animals would experience if they had been able to migrate into deeper water. Still, a rapid decline in the population size of two species, *Asterias rubens* and *Carcinus maenas*, occurred during the winter of 1982–1983, and can be explained as resulting from a combination of low temperatures and a shortage of preferred food in the basins.

Waves and tide

The wave and tide fluctuations of the mesocosms lack the variability found on the shore. The influence of wind and hydrostatic pressure may create a real tidal amplitude in the Oslofjord of about 1 m compared with the regular 0.3 m in the basins. The waves in the fjord also change frequently, in contrast to the basins. The stability of the water regime in the basins created sharply defined borders between zones of total desiccation, periodic air exposure, and total immersion. Because this is the regime under which the organisms with different demands on immersion periods have been established, the sessile part of the community is vulnerable to even slight misadjustment of the water level. This occurred once during the oil experiment in July 1983. A slight lowering of the water level left several granite tiles dry for prolonged periods, causing problems with respect to interpreting data on primary community metabolism (Pedersen 1987).

In rocky-shore mesocosms, therefore, it would be an advantage if wave action and tide range were variable and if stochastic changes were built into the routine regulation of waves and tide.

Community development and deviation

A dominant succession of keystone organisms was demonstrated during the 3 years of community establishment. After the main transplantation in 1979, *M. edulis* settled densely on walls, wave generators, and algae in all basins in 1979 and 1980. This was followed by the appearance of predators *A. rubens* and, slightly later, *C. maenas*, which reduced the *M. edulis* population drastically during 1981–1982. These predators were themselves reduced in number after the summer of 1982, presumably due to a lack of food and low winter temperatures. *L. littorea* and many of the macroalgae (*Cladophora rupestris*, *Fucus distichus* ssp. *edentatus*, *Laminaria saccharina*, *L. digitata*, and *Phymatolithon lenormandii*) showed a gradual increase in density or cover during the community-establishment phase.

Although every effort was made to make the four basins as similar as possible at the time of transplantation, the communities gradually deviated in structure during subsequent years. The species composition and dominant pattern were the same in all basins, but population densities differed for several species, e.g., *F. distichus*, *M. edulis*, and *L. littorea* (Bokn and Moy 1985).

Grazing by *L. littorea* has a strong structuring effect on the algal community in the Oslofjord (Lein 1980). The difference in density of *L. littorea* among the basins was reflected in the densities of its favoured food organism, *Ulva lactuca* (Bokn and Moy 1985) and benthic diatoms (Follum 1985), but not in another potential food algae, *Entermorpha* sp. Moe et al. (1985) explained the differences in density and age structure of *L. littorea* as resulting from *C. maenas* praying on juvenile winkles. The basin with the highest number of crabs had the lowest number of winkles and a dominance of large individuals. Similarly, an inverse relationship between the densities of *M. edulis* and *A. rubens* was indicated in the two control basins (Bokn and Moy 1985). For the other species, basin differences had no observable repercussion on the rest of the community. It must also be stressed that although differences between the control basins made interpreting results difficult in many cases, the differences were not greater than the patchiness characteristic for rocky shores due to changes in topography, inclination, and aspect.

Deviation among replicate communities with time is a general disadvantage in long-term mesocosm experiments and, in most cases, it is unavoidable unless strong population management is executed. Such manipulation is undesirable primarily because it could, in itself, be a significant factor in structuring the community, thereby masking test–control differences of interest. Instead, one should try to prevent deviation through careful analysis of the possible causes in each case, which would then allow one to establish a design that would minimize these factors. In the present rocky-shore mesocosm system, there are several basin differences that might, a priori, cause deviation among communities:

- The structure of the community initially transplanted,
- Microhabitats of the substrate,
- The wave pattern due to slight differences in basin dimensions,
- The input of recruits with the water entering the basin, and
- Temperature differences between the outer and middle basins.

Of these, the differences in microhabitats and recruits have probably been the most significant. The concrete of one of the basins has been damaged more over time than in the other basins, creating a multitude of cracks and crevices. This basin had by far the highest densities of winkles (Bokn and Moy 1985; Lystad and Moe 1985), which has been explained as resulting from the increased shelter for juvenile winkles from predatory shore crabs.

Although self-propagation within the basins may have occurred, most recruits (eggs, larvae, and spores) were brought in with the water. The pump delivers 90 $m^3 \cdot h^{-1}$, of which 10 $m^3 \cdot h^{-1}$ is fed to each basin. The remaining 50 $m^3 \cdot h^{-1}$ goes straight through the pipeline system. Whether this ensures the same supply of organisms to each basin is uncertain. Follum (1985) detected a gradient in input of algal debris and mineral particles from the proximal to the distal basin and suggested that this indicated a difference in the supply of organisms. On the other hand, Lystad and Moe (1985) found no systematic difference in the number of egg capsules of *L. littorea* entering each basin.

Exclosure of organisms

Enclosure also means exclosure of organisms from the basins. The fjord end of the pipeline was equipped with a sieve with 5-mm mesh to prevent larger objects from damaging the pump. This also prevented larger organisms, such as fish, from entering the basins. In addition, the pump could also damage smaller, more delicate organisms. Another reduction in potential recruits was, at times, caused by filter feeders in the pipelines, even though the system was cleaned regularly to prevent fouling. The effect of excluding larger fish from the community is not known, but it is thought to be small, and the exclusion had no direct bearing on the experiment because larger fish were absent from all basins. Smaller flatfish and gobids were present in the basins, but in small numbers.

Plankton hauls at the basin inlet and around the water intake during the summer of 1982 demonstrated that although the water-intake system caused a significant reduction in the number of potential settlers, most species found outside were still present to the water entering the basins (Follum 1984) and in sufficient densities to ensure recruitment.

Indications of diminished recruitment were found, however. Bakke (unpublished data) recorded strong settlement of *M. edulis* on artificial substrates by the water intake during the summer of 1981, and nearly no settlement on the same type of substrate in the basins. Predation was excluded in both areas. Follum (1985) showed that the primary community in the basins was more diatom dominated than on a nearby shore, and concluded that the settling of macroscopic algae and animals was less successful in the basins. The cause for these differences was probably a lack of the stimuli necessary for settlement in the basins, primarily the reduced water movement, rather than a lack of potential settlers. In the header tanks above the basins, where water movement was vigorous, settlement of both mussels and barnacles was comparable to that on the shore, indicating that potential settlers of these and other species were also present in the basins.

Sampling stress

The main size constraint in rocky-shore mesocosms is not that of individual moving space, but that of allowing for population sizes large enough to be adequately sampled without changing the population structure significantly. This again is a function of the scope of the experiment. At Solbergstrand, nondestructive registration was used extensively. With the sessile and essentially two-dimensional structure of a rocky-shore community, this is relatively easy to achieve. Community structure was studied using fixed transects, which permitted recording percentage cover or exact numbers of individuals. Repeated fixed-site photography was used as a basis for establishing the population dynamics of barnacles. Growth was based on measurements of tagged individuals in situ (algae) or of individuals that were later returned to the basins after measurements had been taken (winkles and mussels). Community recruitment and metabolism was studied using replaceable granite tiles (Bokn 1985; Pedersen 1987). In general, destructive sampling was limited to the studies of physiology, biochemistry, population genetics, and the tissue burden of oil. Similar samples were generally removed from all basins to ensure comparability. Sampling loss of the inherent population of *M. edulis* was minimized by using transplanted and caged individuals for physiological and biochemical analyses. *Littorina littorea* suffered the most substantial sampling loss. During the oil experiment, a total of about 600 individuals were removed from each basin for various analytical purposes. This represented from 18 to 80% of the mean population size in each basin (3 358 and 742 individuals for the highest and lowest populated basins, respectively) (Lystad and Moe 1985) and might, therefore, represent a high toll in the basin with the smallest population. However, the population estimates did not suggest the sampling loss to be significant. A gradual reduction in total population of *L. littorea* was recorded in all basins from the summer of 1982 to the summer of 1983, but the least reduction occurred in the basin with the smallest population (Bokn and Moy 1985). Population analysis confirmed that this reduction was independent of sampling loss (Lystad and Moe 1985). The results indicated that sampling must have been balanced by recruitment.

Mesocosms versus field experiments

The cost of establishing and running rocky-shore mesocosms is high in terms of personnel and money. Therefore, one should consider carefully when choosing a mesocosm approach in preference to shore experiments. Such consideration may be based on several aspects: the purpose of the experiment, the type of manipulation involved, sampling and registration techniques to be applied, mesocosm realism, and the duration of community development required before the experiments.

To answer basic questions related to community structure and function, and species interaction, field experiments are preferable to mesocosms because there is no doubt about natural realism in the former. Although population management is more controlled in mesocosms (e.g., removal or introduction of species and population adjustment) and unexpected invasion by predators, such as starfish, fish, and birds, that may disturb field experiments is less likely, satisfactory control with field experiments can also be achieved, e.g., with sufficient personnel.

The problem of replication, i.e., obtaining a sufficient number of comparable experimental communities, is equal in the field and in mesocosms. Both systems will be characterized by short-term structural variability. Also, when using small neighbouring bays of comparable size, the possibility of time-zero differences and gradual deviation with time is similar to that of basins.

Most of the sampling and registration techniques applied in the present mesocosm experiment might, in principle, be applied to equally sheltered shores. For several of the subprojects, a shore reference site was, in fact, included in the investigations. Automatic recording of environmental conditions could also be arranged in situ if necessary. Still, some clear logistic advantages are associated with the use of mesocosms. For instance, experimental installations, such as settling panels, cages, electrodes, etc., are better protected against damage than on a shore with free access. As well, sampling is well controlled. Although a rocky-shore mesocosm is connected to the outside world through the water supply, there is no real exchange of individuals with the surroundings as in field experiments. This allows for excellent control of population sizes. Also, dead individuals with hard structures are usually retained within the mesocosms and can be recorded.

The primary advantage of mesocosms over field experiments remains the opportunity they afford to manipulate water conditions of the ecosystem and to introduce pollutants. In most pollution scenarios of the shoreline, the pollutants are mediated through the water, examples being industrial discharges, oil spills or seeps, cooling water, and freshwater runoff. In a mesocosm, these conditions can easily be replicated, and the pollutant level can be controlled and documented. This is not possible in the field, especially if long-term exposure is desired.

Conclusions

Rocky-shore mesocosms have proven to be a valuable experimental tool in pollution research, and are the only alternative to laboratory experiments if a chronic discharge into coastal water is to be imitated. Mesocosm experiments can also be valuable in basic ecological research on rocky shores, but only under special circumstances are they preferable over field experiments. Important ecological functions, such as competition, predation, recruitment, and seasonality, can be retained in basin communities for several years, and with careful management and advanced technical design, the physical environment can be satisfactorily copied.

The disadvantages of poor experimental replication and deviation of parallel communities over time exist in mesocosms as well as in field experiments, but they are more manageable in the former. However, moderate community deviation must be expected in rocky-shore units, and it should be interpreted as an indication that no strong artificial forcing function is being imposed on the community by the design or the researcher. Even well-designed rocky-shore mesocosms should not be regarded as replicates in laboratory terms, but as a series of reasonably similar and partly independent communities imitating shoreline conditions sufficiently to prevent the inherent organisms from reacting abnormally to the experimental perturbation.

The Solbergstrand mesocosm exercise has provided much experience in this sort of experimentation, and it has suggested several improvements for future development of hard-bottom mesocosms. Better imitation of stochastic events in the physical regime of waves and tides may be obtained through more flexible technology coupled with computer regulation. Community establishment time should be shortened by emphasizing careful transplantation more than water-mediated recruitment. This would also improve structural similarity in replicate communities. Efforts in enclosure techniques for littoral and sublittoral hard-bottom communities should also be pursued to obtain in-situ mesocosms. This would combine field experimental realism with the opportunity to manage the enclosed-water system.

Acknowledgments

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Bakke, T. 1985. Energy balance in *Littorina littorea*. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, December 1984. Norwegian Institute of Water Research, Oslo, Norway. Report 1697-V, 13.1–13.11.

______ 1986. Experimental long term oil pollution in a boreal rocky shore environment. *In* Proceedings of the 9th Arctic Marine Oilspill Programme Technical Seminar. Environment Canada, Ottawa, ON, Canada. 167–178.

Bokn, T. 1985. Effects of diesel oil on commercial benthic algae in Norway. Proceedings of the 1985 Oil Spill Conference. American Petroleum Institute, Washington, DC, USA. 491–496.

Bokn, T., Moy, F. 1985. Community structure. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, December 1984. Norwegian Institute of Water Research, Oslo, Norway. Report 1697-V, 4.1–4.22.

Bonsdorff, E., Nelson, W.G. 1981. Fate and effects of Ekofisk crude oil in the littoral of a Norwegian fjord. Sarsia, 66, 231–240.

Dayton, P.K. 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. Ecological Monographs, 45, 137–159.

Fevolden, S.E., Garner, S.P. 1986. Population genetics of *Mytilus edulis* (L.) from Oslofjorden, Norway, in oil-polluted and non oil-polluted water. Sarsia, 71, 247–257.

Follum, O.-A. 1984. A comparison between an artificial locality at Solbergstrand Field Station (Basin II) and a natural locality with respect to settlement and growth — A summary. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, December 1983. Norwegian Institute of Water Research, Oslo, Norway. Report 1578-IV, 14.1–14.36.

______ 1985. Settlement, growth and community structure on granite chips: a comparison between four basins at S.E.S. and a sheltered locality in the Oslofjord. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, December 1984. Norwegian Institute of Water Research, Oslo, Norway. Report 1697-V, 6.1–6.11.

Gray, J.S. 1987. Oil pollution studies of the Solbergstrand mesocosms. Philosophical Transactions of the Royal Society, London (Series B, Biological Sciences) 316, 641–654.

Lein, T.E. 1983. The effects of *Littorina littorea* L. (Gastropoda) grazing on littoral green algae in the inner Oslofjord, Norway. Sarsia, 65, 87–92.

Livingstone, D.R., Moore, M.N., Lowe, D.M., Naschi, C., Farrar, S.V. 1985. Responses of the cytochrome P-450 monooxygenase system to diesel oil in the common mussel, *Mytilus edulis* L., and the periwinkle, *Littorina littorea* L. Aquatic Toxicology, 7, 79–91.

Lowe, D.M., Pipe, R.K. 1985. Cellular responses in the mussel *Mytilus edulis* following exposure to diesel oil emulsions: reproductive and nutrient storage cells. Marine Environmental Research, 17, 234–237.

Lystad, E., Moe, K. 1985. Comparison of population dynamics, growth and reproductive cycle in *Littorina littorea* (L.) at four artificial and one natural locality in Solbergstrand, Oslofjorden, where two of the artificial localities were exposed to hydrocarbons. University of Oslo, Oslo, Norway. Cand. Real. degree thesis. 198 pp.

Moe, K.-A., Follum, O.-A., Lystad, E. 1985. On *Carcinus maenas* (L.) in the four rock bottom basins at Solbergstrand Experimental Station. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, 1985. Norwegian Institute of Water Research, Oslo, Norway. Report 1809-VI, 32–35.

Moore, M.N., Livingstone, D.R., Lowe, D.M. 1985. Sublethal cellular and molecular effects and short term recovery of mussels (*Mytilus edulis*) and periwinkles (*Littorina littorea*) following chronic exposure to petroleum hydrocarbons. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, December 1984. Norwegian Institute of Water Research, Oslo, Norway. Report 1697-V, 14.1–14.37.

Notini, M., Nagell, B., Hagstrom, A., Grahn, O. 1977. An outdoor model simulating a Baltic Sea littoral ecosystem. Oikos, 28, 2–9.

Pedersen, A. 1985. Community metabolism in fouling assemblages. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, 1985. Norwegian Institute of Water Research, Oslo, Norway. Report 1809-VI, 14–16.

______ 1987. Community metabolism on rocky shore assemblages in a mesocosm — A: Fluctuations in production, respiration, chlorophyll *a* content and C:N ratios of grazed and non-grazed assemblages. Hydrobiologia, 151/152, 267–275.

Sporstol, S., Oreld, F. 1985. Chemical analysis of hydrocarbon content in organisms. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, December 1984. Norwegian Institute of Water Research, Oslo, Norway. Report 1697-V, 3.1–3.11.

Thome, P., Walday, M. 1984. *Mytilus edulis*, population structure and dynamics. *In* Bakke, T., ed., Long term effects of oil on marine benthic communities in enclosures: progress report, December 1983. Norwegian Institute of Water Research, Oslo, Norway. Report 1578-IV, 10.1–10.9.

Widdows, J., Donkin, P., Evans, S.V. 1985. Recovery of *Mytilus edulis* L. from chronic oil exposure. Marine Environmental Research, 17, 250–253.

Benthic Mesocosms in the Netherlands

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In the mid-1970s, two indoor mesocosms, mimicking an intertidal mudflat environment, were built. Ten years of observations and experimentation in these facilities showed that the structure and major functions of the present experimental ecosytems behaved in a very realistic way. The systems were largely selfmaintaining and had a long lifespan. Consequently, eight similar mesocosms were built in the open air and used in applied studies into the ecological effects of oil spills and harbour sludge in coastal areas on mudflat ecosystems. The results contributed in a significant way to methods of dealing with such perturbations and, moreover, provided better insight into the functioning of ecosystems in general.

Research on marine mesocosms in the Netherlands dates back to the early 1970s, wooden enclosures were erected around natural mussel beds in the western Wadden Sea and were used in pollution studies (de Wolf et al. 1972).

In 1975, both the introduction of large plastic bags for pollution studies in coastal waters (Kuiper 1977) and the construction of two large indoor mesocosms (de Wilde and Kuipers 1977) for fundamental research in intertidal mudflats followed. The indoor systems were used with changing time intervals for a period of almost 10 years. From the results obtained, it was concluded that benthic mesocosms were a new and promising tool for ecological studies.

In 1980, four model tidal flats (MOTIFs) were established in the open air (Kuiper et al. 1983, 1984). Three years later, another four MOTIFs were built. From 1981 until 1987, under the umbrella of OPEX[1], applied research has been carried out on the short- and long-term effects of oil pollution on intertidal mudflat areas and on oil-combatting techniques in the Dutch Wadden Sea. In 1987, under the acronym SEDEX (Sediment Experiment), experiments on the effects of dumping harbour sludge were started. An overview of mesocosm research in the Netherlands is shown in Fig. 1. As the marine enclosure experiments with plankton communities in plastic bags have already been described by Kuiper (1982), only

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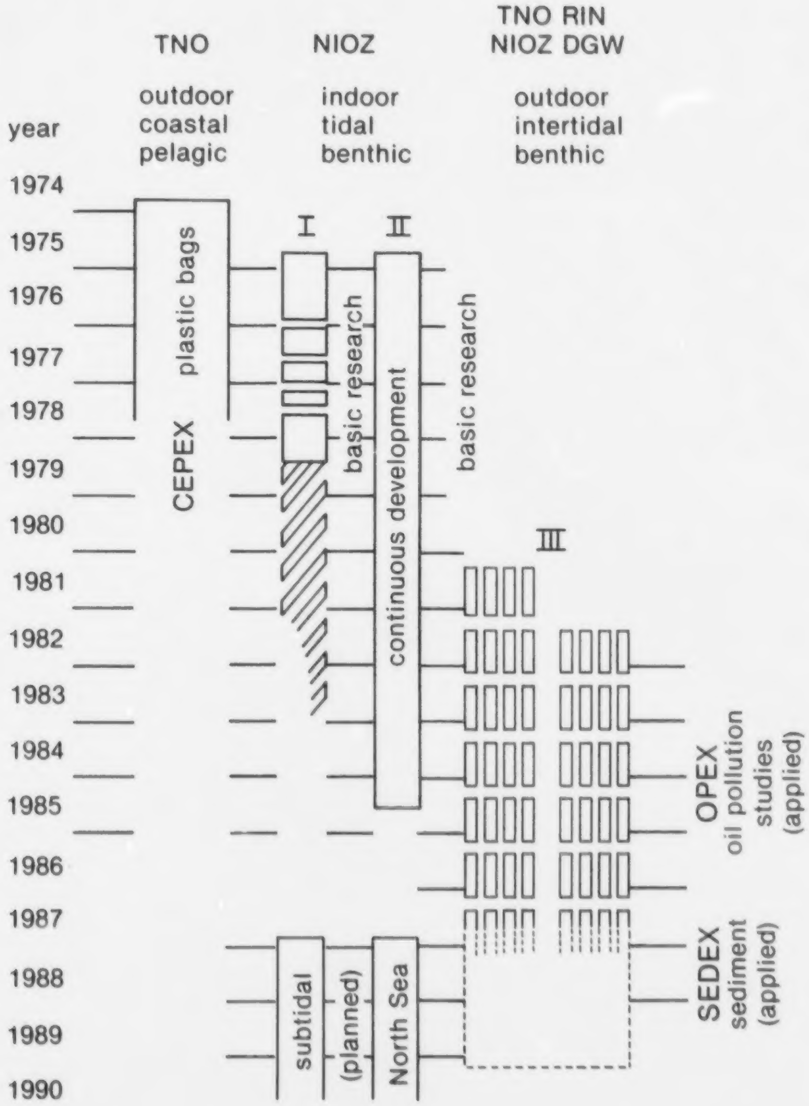
[1] OPEX (Oil Pollution Experiment) is a cooperative research program of the Netherlands Organization for Applied Scientific Research (TNO), Division of Technology for Society in the Netherlands, the Netherlands Institute for Sea Research (NIOZ), the Research Institute for Nature Management (RIN), and the Department of Tidal Waters (DGW) of the Ministry of Public Works and Waterways.


TNO
NIOZ
TNO RIN
NIOZ DGW
year
outdoor coastal pelagic
indoor tidal benthic
outdoor intertidal benthic
1974
1975
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1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
plastic bags
CEPEX
I
II
III
basic research
continuous development
basic research
OPEX
oil pollution studies (applied)
SEDEX
sediment (applied)
subtidal
(planned)
North Sea

Fig. 1. Overview of mesocosm research in the Netherlands. Two indoor mesocosms were used over a period of 10 years for fundamental research and eight outdoor model tidal flats were used over a period of 7 years for applied studies dealing with oil pollution and dumping harbour sludge. Subtidal North Sea mesocosms are under construction. Acronyms: TNO, Netherlands Organization for Applied Scientific Research; NIOZ, Netherlands Institute for Sea Research; RIN, Research Institute for Nature Management; DGW, Department of Tidal Waters of the Ministry of Public Works and Waterways; CEPEX, Controlled Ecosystem Pollution Experiment; OPEX, Oil Pollution Experiment; and SEDEX, Sediment Experiment.

the results from the indoor benthic systems are discussed here. Some results from the OPEX experiments are also referred to.

What is a benthic mesocosm?

A benthic mesocosm is an artificial benthic ecosystem on a mesoscale (cf., Banse 1982; Grice and Reeve 1982). It contains a variety of primarily benthic organisms belonging to various trophic levels, showing mutual relations, comparable to those found in nature (Ringelberg 1976). Basic ecological functions, i.e., primary and secondary production and mineralization, must occur in a realistic way. A benthic mesocosm must exhibit an unchanging or predictable state for relatively long periods of time. Human interference must be kept to a minimum.

Indoor mesocosms

The two indoor mudflat ecosystems, each with a surface area of 25 m^2, provided fully controlled systems with regard to energy input, tidal regime, and temperature (for a detailed description, see de Wilde and Kuipers (1977)). One of these mesocosms (I) was used to accommodate a number of divergent experiments pertaining to scientific problems of current interest. The other mesocosm (II), after it was set up with sediment and organisms, was left undisturbed for almost 10 years. The evolution of the benthic structure and the energy flow through the latter system were monitored. Some of the results from both the indoor mesocosms and the outdoor MOTIFs are now used to illustrate the variety of applications and potential of artificial benthic ecosystems.

Growth of the lugworm

During the last few decades, an increasing phosphate load, originating from river discharges, has caused eutrophication and phytoplankton bloom formation in the coastal waters of the North Sea. A considerable part of the organic matter produced here ultimately entered the Wadden Sea and gave rise to enhanced food availability on the tidal mudflats. The effect of extra food on this intertidal ecosystem, with a macrofauna biomass of ±27 g ash-free dry weight (AFDW) (Beukema 1976), was unknown. Laboratory and mesocosm experiments showed that growth in selected faunal elements of the mudflats could easily cope with the extra supply of food by speeding up growth (de Wilde and Berghuis 1979).

Based on the growth curves for the lugworm *Arenicola marina* (Fig. 2), it was concluded that growth of *Arenicola* in its natural habitats was far below its potential. The macrofauna biomass in the mesocosm reached a value of ±50 g $AFDW \cdot m^{-2}$. Ten years later, macrofauna biomass in the Wadden Sea had increased from 27 to 40 g $AFDW \cdot m^{-2}$ (de Wilde and Beukema 1984). This led to the striking conclusion that the intertidal ecosystem of the Wadden Sea is apparently not predator controlled (Reise 1977), but seriously food limited.

Formation of the stratified layer

The bioturbation activity of lugworms is well known. Subterraneous sediment uptake, downward transport of sediment particles in the feeding shaft, upward particle transport by the animal itself, and deposition of the feces at the sediment

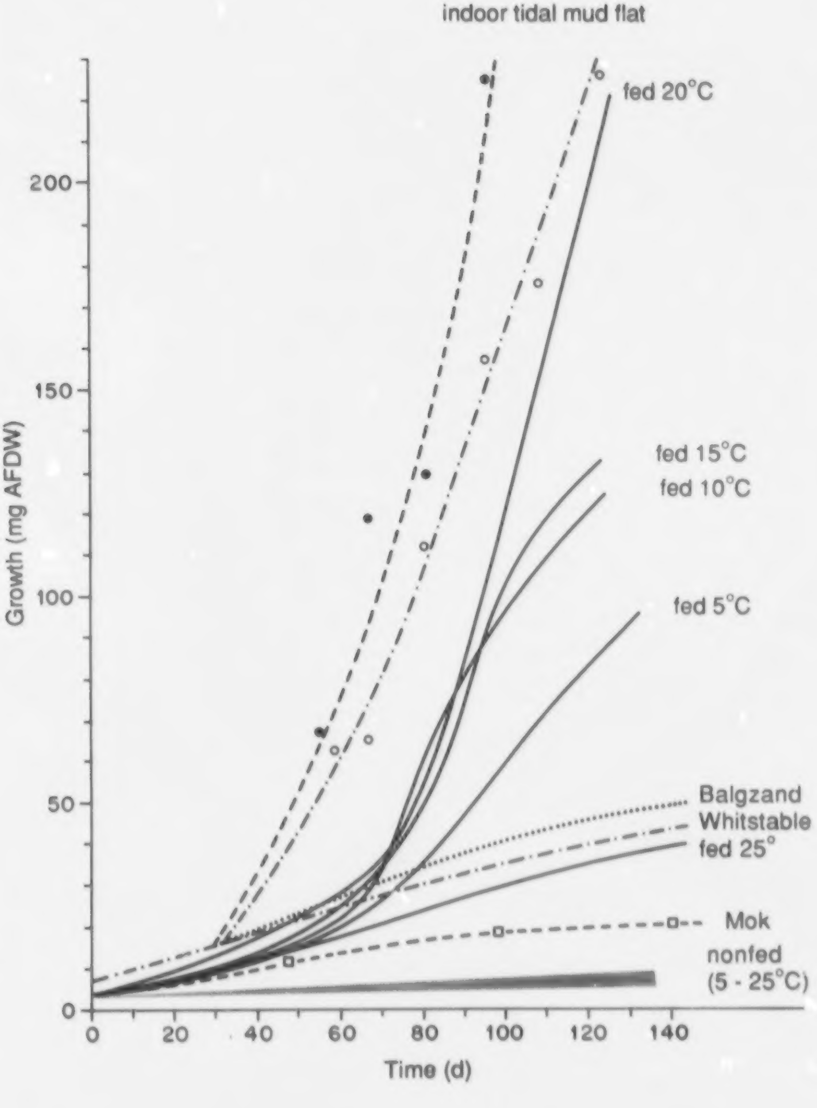

Fig. 2. Food limitation in macrobenthic organisms in natural mudflat systems in the Dutch Wadden Sea. Growth curves (solid curves) obtained from laboratory experiments for fed *Arenicola marina* (Polychaeta) at different temperatures and for nonfed animals (shaded bar) are compared with the growth of juvenile *Arenicola* under field conditions at a nursery area at Mok Bay, Texel (open squares) and at the Balgzand area (dotted line) (Beukema and de Vlas 1979); at the Whitstable flats, UK (Newell 1948); and in two indoor mudflats (open and solid circles) (de Wilde and Berghuis 1979).

surface causes stratification in the sediment (Straaten 1952), with a layer of coarse particles (shell fragments, peat lumps, etc.) at the living depth of *Arenicola*. Natural flats are exposed to tidal currents and wave action, and the time scale within which such layers are formed in the sediment is poorly understood.

In the indoor mesocosm, filled with homogenized mudflat sediment, the number and size of the lugworms was known exactly. The formation of stratified layers in the sediment generated by lugworms was monitored for 400 days. Figure 3 shows the development of heterogeneity in the sediment (Baumfalk 1979).

Mineralization in the sediment

In July 1976, the vertical distribution of mineralization processes in the sediment of the mesocosm was studied (Vosjan and Olanczuk-Neyman 1977). The values for the profiles of the organic carbon content, the sulphate–chlorinity ratio and the sulphide content of the interstitial water, oxygen utilization, and electron transport system (ETS) activity all appear to be sufficiently realistic when compared with certain parts of natural mudflat sediments (Fig. 4).

Effect of the larger predators

Toward the end of November 1977, 12 small flatfish (*Solea solea*), averaging 13 cm in length, were released into the mesocosm. Within a couple of weeks, the rich polychaete stocks of the mesocosm were completely exterminated by the sole, a notorious predator of worms. The numbers of *Arenicola* had decreased from 60 to <10 specimens·m^{-2}; those of *Nereis diversicolor* from about 5 000 to <1 000 specimens·m^{-2}. It is concluded that introducing larger predators into mesocosms should be considered very carefully. In most cases, the scale of the mesocosm may be insufficient to sustain the needs of the predator, depending on the size of the mesocosm.

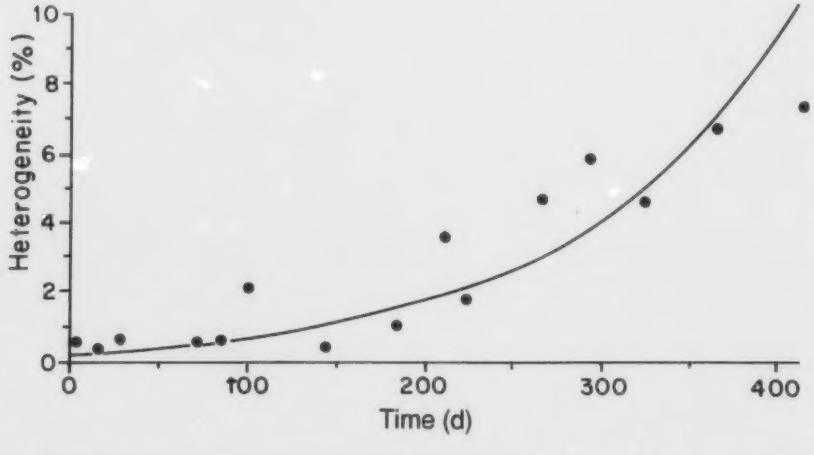

Fig. 3. Development of heterogeneity (%) of median particle size in an indoor tidal-flat mesocosm (Baumfalk 1979).

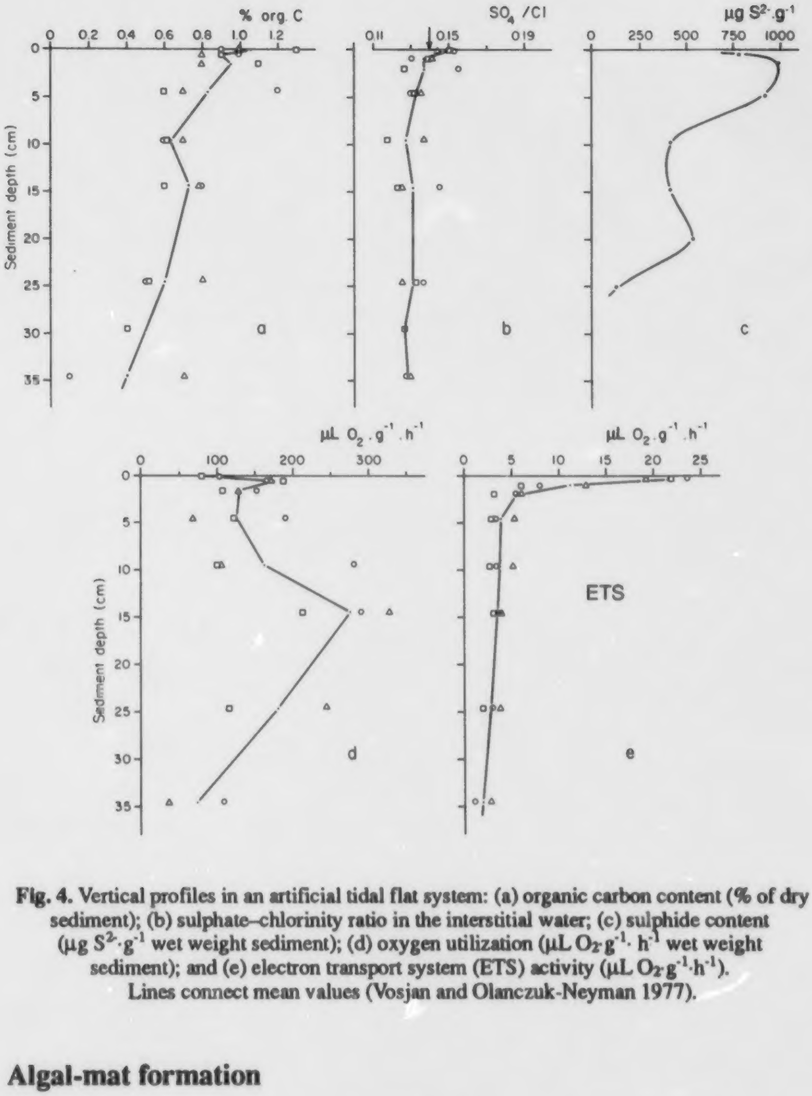

Fig. 4. Vertical profiles in an artificial tidal flat system: (a) organic carbon content (% of dry sediment); (b) sulphate–chlorinity ratio in the interstitial water; (c) sulphide content (μg $S^{2-}\cdot g^{-1}$ wet weight sediment); (d) oxygen utilization (μL $O_2\cdot g^{-1}\cdot h^{-1}$ wet weight sediment); and (e) electron transport system (ETS) activity (μL $O_2\cdot g^{-1}\cdot h^{-1}$). Lines connect mean values (Vosjan and Olanczuk-Neyman 1977).

Algal-mat formation

The occurrence of algal-mat structures on the tidal flats in the Wadden Sea during the summer is a common phenomenon. Suddenly, the mudflats become covered with dense layers of filamentous and thallus, green and blue-green algae, which asphyxiate the infauna. An explanation for the edaphic and sudden appearance of the algal mats is lacking. In an attempt to understand the development of these mats, a series of experiments and observations was carried out (B. Kuipers, unpublished data). To this end, the mesocosm was rearranged in the summer of 1978. The existing system was removed and the basin was subdivided into eight compartments. Each compartment was filled with sterilized sandy sediment, low in silt content and poor in organic matter and nutrients. Initially, the system was kept in the dark. On 15 September 1978, a 2-L sample of the upper sediment layer, scraped from a natural mudflat, was spread over the mesocosm. Previously, this sample was treated with an organic phosphorus compound (parathion), which killed the higher organisms but spared the plant material. Key nutrients in the 40 m^3 of seawater belonging to the system were brought up to concentrations representative of the Wadden Sea in summer: 47 μM NO_3, 21 μM SiO_4, and 7 μM PO_4.

Changes in nutrient concentrations in the mesocosm were recorded weekly. Later, on two other occasions, nutrient concentrations were raised again to the starting level. The experiment started when the lights over the mesocosms were turned on.

The developing vegetation consisted predominantly of *Enteromorpha* and *Chaetomorpha* species, which soon covered most of the sediment. The biomass of the algae was measured weekly in random collections of algae from 400 cm^2 surface areas. The material was dried (65°C) and combusted (2 h at 520°C).

The phytobiomass production in this ungrazed experimental situation had an exponential increase during the first 45 days (Fig. 5). The biomass doubled every 4–5 days, i.e., a daily increase of 18–20%. Of the nutrients in the mesocosm, nitrogen showed an almost reciprocal trend to that of the algae biomass, decreasing from the initial value of 47 μM to a value between 15 and 20 μM after 45 days, at which point it obviously started to limit the growth of the dominating algal population. After this first outburst, the threadlike algae became covered with diatoms, causing the colour of the algal mat to change to yellow. The increase in biomass between 50 and 60 days is attributed to these diatoms. Ultimately, a total biomass of about 11 $g\ C \cdot m^{-2}$ was reached (Fig. 6). On day 67, nutrient concentrations were again brought up to the initial values. Nine days later, this had led to a total biomass of 25.4 $g\ C \cdot m^{-2}$, after which the same nutrient treatment once again raised the biomass to 45 $g\ C \cdot m^{-2}$ over the next 40 days. During the later blooms, however, a considerable population of the grazing isopod *Jaera albifrons* had developed in the mesocosm; hence, only the first 40 days of the experiment can be considered to have provided a production estimate through a biomass increase. Thus, over a period of about 4 months, a total phytobiomass of 45 $g\ C \cdot m^{-2}$ had been produced. In light of the grazing activities of *Jaera*, this value clearly represents an underestimate. Unfortunately, the biomass that was grazed could not be measured.

Fate of organic matter in ecosystems

The structure and composition of organic matter determines whether this food can be utilized for maintenance, growth, and reproduction of organisms. Commonly, organic matter in ecological studies is evaluated in terms of organic carbon or organic nitrogen, but this presents a very rough indication and largely ignores the molecular structure and quality of the available food (Boon and Haverkamp 1979). Pyrolysis mass spectrometry (Meuzelaar et al. 1977) presents a technique by which organic matter is degraded and the fragments obtained are "fingerprinted" by mass spectrometry.

Studying the fate of newly produced organic matter in a mesocosm offers a unique opportunity to follow the pathways of food particles in marine ecosystems.

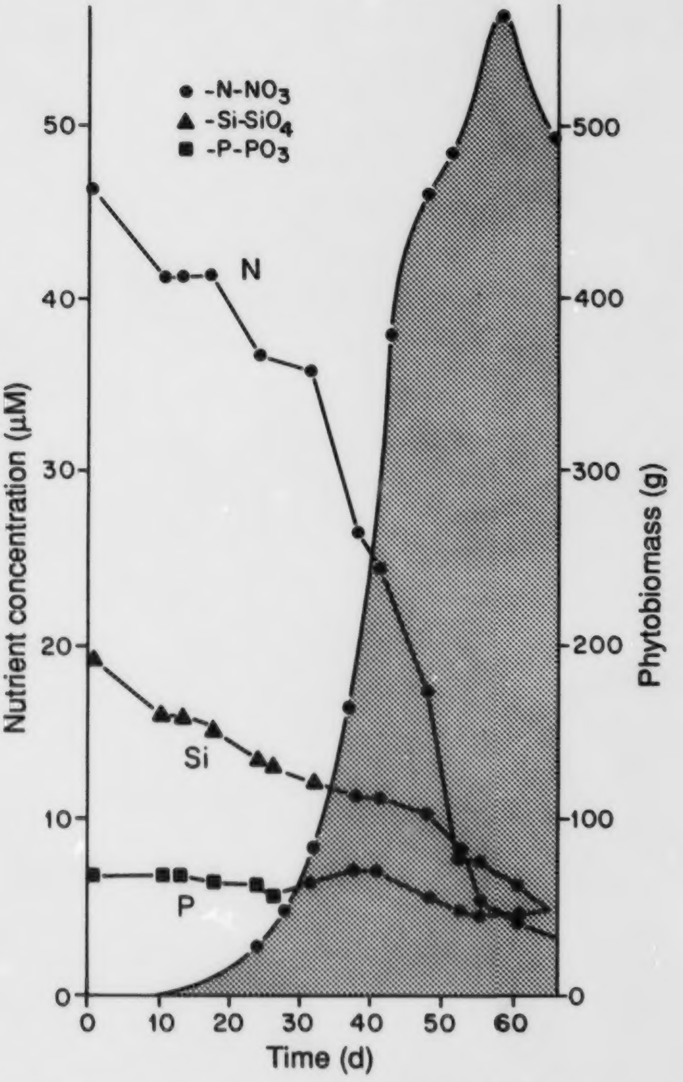

Fig. 5. Production of algal-mat biomass (g ash-free dry weight (AFDW) per 20 m^2) following the supply of nutrients (NO$_3$, SiO$_4$, and PO$_4$) to an intertidal mudflat mesocosm (B. Kuipers, unpublished data).

In a similar study, Boon and Haverkamp (1979) studied the degradation and mineralization of organic matter, both aerobic and anaerobic, mediated by polychaete worms in the mesocosm. Mass pyrograms were obtained from fresh algae, fecal pellets, and aerobic and anaerobic sediments (Fig. 7).

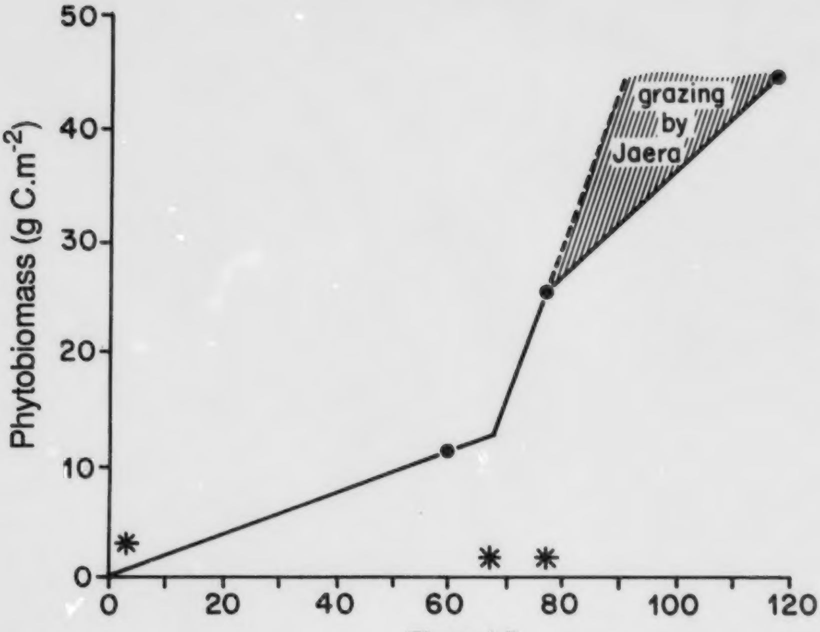

Fig. 6. Development of total phytobiomass (g C·m^{-2}) by algal-mat structures in the indoor mesocosm. The shaded area represents the estimated biomass grazed by *Jaera*. Asterisks indicate nutrient additions (B. Kuipers, unpublished data).

Development of an experimental mudflat ecosystem

As already mentioned, the second indoor mesocosm was left almost undisturbed for about 10 years.

Faunal assemblage

In 1975, the system, with 200 lugworms·m^{-2}, was initially completely dominated by the polychaete worm *Arenicola marina*. The growth of this species in the mesocosm was discussed earlier. Notwithstanding the complicated larval and juvenile development of *Arenicola* (Farke and Berghuis 1979), the species was able to complete its reproductive cycle in the mesocosm. However, a steady decline in population density was observed, with values of 85 lugworms·m^{-2} in 1976 and 20 lugworms·m^{-2} in 1980 (Fig. 8). In 1985, lugworms had almost vanished, with numbers decreasing to <5 lugworms·m^{-2}. This reduction was due to a rapidly increasing population of a more opportunistic worm species, *Nereis diversicolor*. *Nereis* was introduced in error into the mesocosm, probably with the juvenile lugworms. In the mesocosm, *Nereis* was able to reproduce several times a year (F. Witte, unpublished data; W. Wiersinga, unpublished data). The species proved to be a competitor for algae. In addition, the adult *Nereis* is a serious predator of juvenile lugworms (Witte and de Wilde 1979).

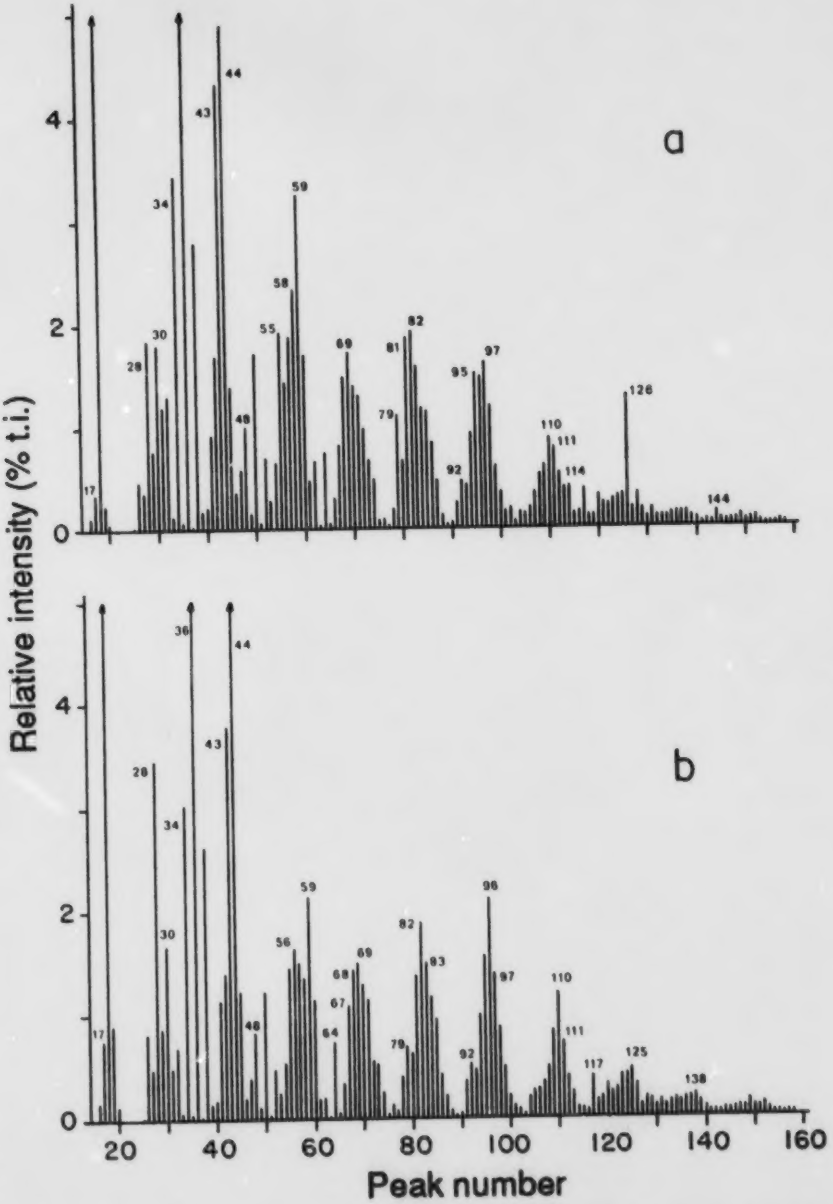

Fig. 7. Curie-point pyrolysis mass spectra of surface sediment layers from an experimental laboratory-scale marine ecosystem. (a) Aerobic upper-surface sediment (algal mat) at 0–3 mm depth. (b) Anaerobic upper surface sediment (black mud) at 3–10 mm depth. The height of the peaks is expressed in arbitrary units, i.e., as a percentage of the total count intensity (t.i.) (Boon and Haverkamp 1979).

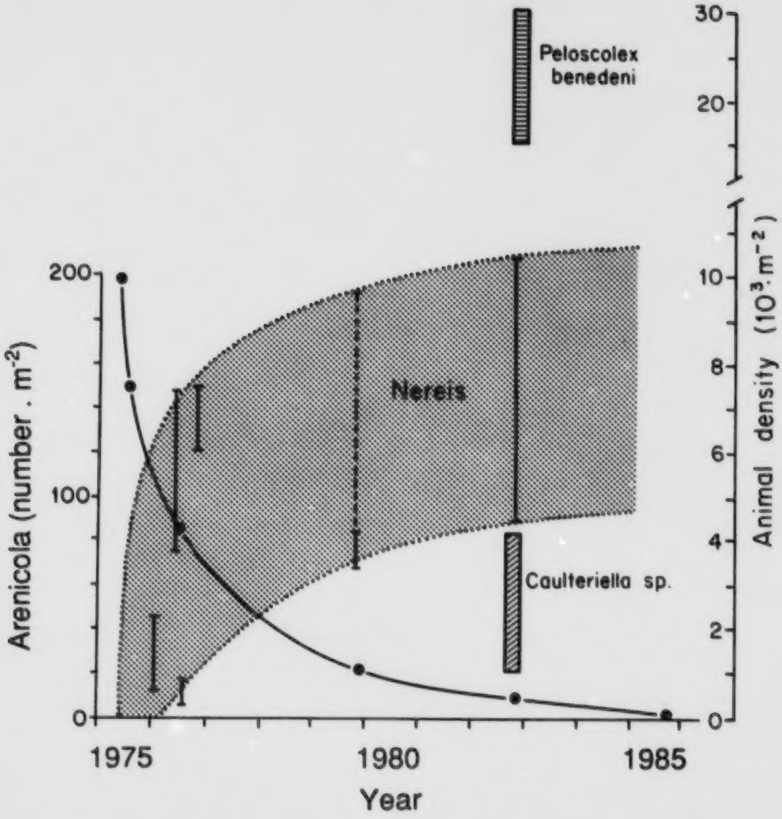

Fig. 8. Development of macrobenthic worm populations in an intertidal mudflat mesocosm. More opportunistic species (*Nereis*, *Caulteriella*, and *Peloscolex*) take over, which is detrimental to *Arenicola*. Bars indicate the spread in the estimated numbers. Shaded area presents the estimated limits in which the population of *Nereis* fluctuated. *Caulteriella* and *Peloscolex* were only studied in 1982.

During further development of the mesocosm, the *Nereis* population maintained a very high density, ranging from 3 000 to more than 10 000 specimens·m^{-2}. Finally, during the last years of the existence of the mesocosm, large numbers (1 000–4 200 specimens·m^{-2}) of the small polychaete worm *Caulteriella* species and of the oligochaete worm *Peloscolex benedeni* were present. Meiofauna organisms, mainly nematodes, numbered between 1 and 2 million organisms·m^{-2} over the period in which the mesocosm existed.

Biomass

During the 1st year, the total macrofauna biomass in the mesocosm reached about 50 g AFDW·m^{-2} of which 80% was contributed by *Arenicola* (Fig. 9). Four years later, the biomass measured only 32 g AFDW·m^{-2} and, in 1982, it measured

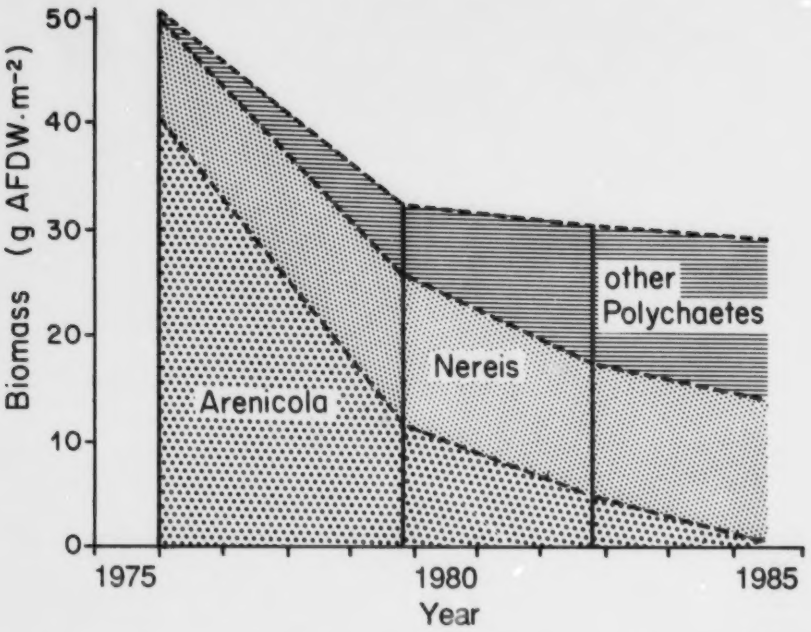

Fig. 9. Development of macrobenthic biomass (g ash-free dry weight (AFDW) per m^2) in an intertidal mudflat mesocosm, stabilizing at 30 g AFDW·m^{-2}.

30 g AFDW·m^{-2}. Apparently, the biomass had adjusted to a level related to the amount of food available in the mesocosm. The share of *Arenicola* with respect to the total biomass had decreased gradually, that of *Nereis* remained unchanged, and other opportunistic species increased. The growth of opportunistic species provides evidence of the disturbed character of the mesocosm.

Energy flow

To obtain insight into the relationship between the standing stocks of macrofauna organisms and the availability of food, energy flow budgets were developed. The input of light energy into the system was well documented. Irradiance, 12 h·d^{-1} produced by ten 400-W sodium bulbs, was periodically measured at the sediment surface during both low and high tide. Initially, during the 1st year, the irradiance amounted to $\pm 1.4 \times 10^9$ J·m^{-2}·a^{-1}. Then, to obtain a more homogeneous light distribution, the light sources were suspended at a higher level, and the irradiance decreased to $\pm 0.4 \times 10^9$ J·m^{-2}·a^{-1} (Fig. 10). The light input in the mesocosm was low.

On three occasions, the rates of primary production and mineralization, both in the water and in the sediment, were measured (Table 1). Some comparable data are also included from the outdoor mesocosms and from the natural flats. Most obvious is the extremely high level of primary productivity in the mesocosm, particularly when the low light levels are taken into account. The rather uniform ambient factors must have facilitated the selection of algae that can grow efficiently under

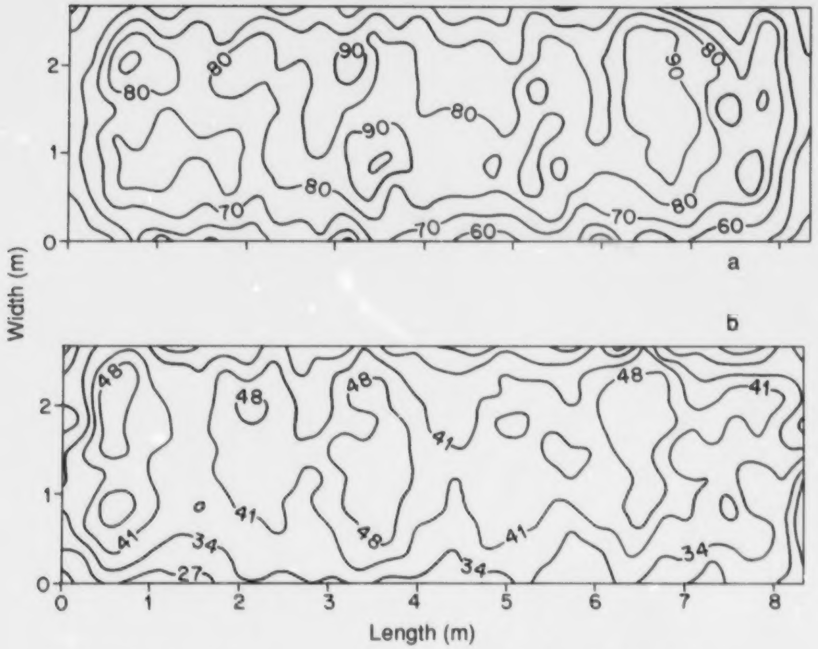

Fig. 10. Light irradiance (microamperes, µA) at the sediment surface of the indoor mesocosm: (a) during low water, mudflat exposed, the interval between isolines is 10 µA; (b) during high water, 50-cm water depth, the interval between isolines is 7 µA (Hofstede and Stelder 1980).

such low-light conditions. Moreover, it is known that for diatoms, light saturation values may be as low as 10% of natural sunlight.

A second remarkable feature is the relatively high biomass in the mesocosms. A gradual shift was expected to a system dominated by microorganisms and small food-web organisms (meiofauna and protozoans); instead, the macrofauna, with a total biomass ranging from 30 to 50 g AFDW·m^{-2}, maintained high densities. In the outdoor mesocosms (discussed below), and in the natural environment of the Wadden Sea, equally high biomass is found (Dekker and van Moorsel 1987).

Some information on mineralization activity in the mesocosm (derived from oxygen demand in both sediment and water) was obtained on two occasions. In 1979, only 10% of the available carbon was aerobically mineralized in the water column and 30% in the sediment. Apparently, the rest was degraded anaerobically or was buried in the sediment. In 1983, much higher mineralization, i.e., 50% of the organic carbon, occurred in the water, and about the same percentage in the sediment. The notable increase of mineralization in the water is related to the gradual enhancement of particulate and dissolved organic carbon in this part of the system. In contrast to the relatively small residence times of the water in the outdoor mesocosms and in the Wadden Sea, i.e., 5–7 days, no exchange of water occurs in the indoor mesocosm.

Table 1. Tentative energy flow in the intertidal mesocosms and in the western Wadden Sea (de Wilde and Beukema 1984).

	Indoor mesocosms			MOTIFS (1983–1985)	Outdoor natural mudflat, Wadden Sea
	1975–1976	1979	1983		
Energy input ($MJ \cdot m^{-2} \cdot a^{-1}$)	1400	400	400	3800	3800
Primary production ($g\ C \cdot m^{-2} \cdot a^{-1}$)	200	400	300	180	150
Extra supply ($g\ C \cdot m^{-2} \cdot a^{-1}$)	?	—	—	—	230
Total "food" available ($g\ C \cdot m^{-2} \cdot a^{-1}$)	200	400	300	180	380
Macrobenthic biomass ($g\ AFDW \cdot m^{-2}$)	50	30	35	25–35	30–40
Mineralization ($g\ C \cdot m^{-2} \cdot a^{-1}$)					
Pelagic	?	40	165	70	40
Benthic aerobic	?	120	165	110	260
Benthic anaerobic	?	?	?	?	80
Burial	?	?	?	?	?

Notes: Macrobenthic biomass in the various systems tends toward a comparable value of 30–40 g ash-free dry weight (AFDW) per m^2. The natural mudflats of the Wadden Sea have an extra supply of organic matter from the North Sea. Food available means available for pelagic and benthic food-chain organisms and for mineralization by microorganisms. Question marks indicate a lack of observation; dashes indicate negligible amounts.

Outdoor benthic mesocosms

Model tidal flats were used successfully during the last 5 years in applied research dealing with the effects of oil pollution and with oil-combatting techniques in the Wadden Sea (Kuiper et al. 1986). As a direct effect of (dispersed) oil or dispersant, or both, and in relation to their concentrations, distinct changes in behavioural activities and also (mass) mortalities in certain susceptible mudflat organisms in the mesocosms were observed. Moreover, it could be demonstrated that a considerable part of the oil stranded on the mudflats is buried in the sediment, mainly by the bioturbative activities of lugworms.

Buried oil can seriously disturb normal development and regeneration of the mudflat ecosystem over extended periods. Thus, the model tidal flat experiments showed not only the short-term effects (mortality) but also some unexpected long-term effects. The results of the experiments contributed significantly to oil-combatting strategy in the Wadden Sea.

The experiments also led to the identification of indicator species. The behaviour of these species is indicative of the effect of pollution on the entire ecosystem. *Corophium volutator*, a small amphipod, was considered to be a good indicator. Its numbers and population development reflected very well, and in a dose-dependent way, the extent of the artificial oil spills in the mesocosms.

It is emphasized here that mesocosms used in applied studies will also provide fundamental knowledge of the functioning of ecosystems in general; in this case, in terms of larval settling, species interaction, and growth. Controls, running together with the contaminated systems, fulfill a key function. The more or less comparable total biomass, observed in differently treated mesocosms, indicated that other species easily compensate for reduced secondary production by speeding up growth in case certain susceptible species are reduced in numbers.

Conclusions

The main objective of this paper was to document the usefulness of benthic mesocosms. The investigator must decide on the proper mesoscale of the marine ecosystem to be tested. The design, intended structure of the benthic ecosystem, and expected lifespan of the mesocosm are primarily dictated by the kind of scientific questions that are asked.

In general, mesocosm experiments are thought to provide, and facilitate, a special type of research, such as ecotoxicology and selected management problems, and to combat pollution, in which they will bridge the gaps between laboratory and field experiments. Mesocosm experiments are certainly not intended as a substitute for marine research in the field.

The dimensions of most mesocosms are set pragmatically, rather than being based on sound arguments. Benthic mesocosms mimicking the intertidal mudflat environment do not offer special problems. The associated organisms are hardy and able to resist large variations in ambient conditions. Mesocosms that will properly mimic the benthic system of shelf seas, or the even more difficult open ocean, still pose a great challenge.

It is a misunderstanding that budgets associated with mesocosm research are much smaller than those associated with similar research in nature. Indeed, logistics are sometimes easier in mesocosms, and disturbances and calamities caused by external factors, such as the weather, may be reduced or excluded. On the other hand, the various relationships and processes taking place in a well-developed mesocosm are as complicated as those occurring in nature. Adequate monitoring of the dynamics of the dominant species or groups in the mesocosm and of the key functions of the system is laborious and will require the attention of a multidisciplinary team of scientists.

Acknowledgments

Much of the research in the indoor mesocosms was carried out with the cooperation of the following students: Jacqueline van der Hofstede, Yvonne van Scheppingen, Chris Stelder, Wim Wiersinga, and Frans Witte. Mr Eilke Berghuis offered skilful technical assistance. Special thanks are due to my colleague Dr Bouwe Kuipers for putting part of his unpublished results on algal mat production at my disposal.

Banse, K. 1982. Experimental marine ecosystem enclosures in a historical perspective. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 11–24.

Baumfalk, Y.A. 1979. Heterogeneous grain size distribution in tidal flat sediment caused by bioturbation activity of *Arenicola marina* (Polychaeta). Netherlands Journal of Sea Research, 13, 428–440.

Beukema, J.J. 1976. Biomass and species richness of the macrobenthic animals living on the tidal flats of the Dutch Wadden Sea. Netherlands Journal of Sea Research, 10, 236–261.

Beukema, J.J., de Vlas, J. 1979. Population parameters of the lugworm *Arenicola marina*, living on tidal flats in the Dutch Wadden Sea. Netherlands Journal of Sea Research, 13, 331–353.

Boon, J.J., Haverkamp, J. 1979. Pyrolysis mass spectrometry of a benthic marine ecosystem — the influence of *Arenicola marina* on the organic matter cycle. Netherlands Journal of Sea Research, 13, 457–478.

Dekker, R., van Moorsel, W.N.M. 1987. Effects of different oil doses, dispersant and dispersed oil on macrofauna in model tidal flat ecosystems. *In* Kuiper, J., van den Brink, W.J., ed., Fate and effects of oil in marine ecosystems. Nijhoff, Dordrecht, Netherlands. 117–131.

De Wilde, P.A.W.J., Berghuis, E.M. 1979. Laboratory experiments on growth of juvenile lugworms, *Arenicola marina*. Netherlands Journal of Sea Research, 13, 487–502.

De Wilde, P.A.W.J., Beukema, J.J. 1984. The role of the zoobenthos in the consumption of organic matter in the Dutch Wadden Sea. Netherlands Institute for Sea Research, Texel, Netherlands. Publication Series 10-1984, 145–158.

De Wilde, P.A.W.J., Kuipers, B.R. 1977. A large indoor tidal mud-flat ecosystem. Helgolaender Wissenschaftliche Meeresuntersuchungen, 30, 334–342.

De Wolf, P., de Kock, W.Chr., Stam, A. 1972. Field experiments on the influence of copper and mercury in a natural mussel bed. Netherlands Organization for Applied Science Research — Nieuws 27, 497–504.

Farke, H., Berghuis, E.M. 1979. Spawning, larval development and migration behaviour of *Arenicola marina* in the laboratory. Netherlands Journal of Sea Research, 13, 512–528.

Grice, G.D., Reeve, M.R. 1982. Introduction and description of experimental ecosystems. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 1–9.

Hofstede, J. van der, Stelder, C. 1980. Stofkringloop en energiehuishouding in wadbak zuid. Netherlands Institute for Sea Research, Texel, Netherlands. Internal Report, 1980-10, 1–42. [Summary in English.]

Kuiper, J. 1977. Development of North Sea coastal plankton communities in separate plastic bags under identical conditions. Marine Biology, 44, 97–107.

______ 1982. Ecotoxicological experiments with marine plankton communities in plastic bags. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 181–193.

Kuiper, J., van het Groenewoud, H., Hoornsman, G., de Wilde, P., Berghuis, E., Kok, A., Wolff, W., Schoonheden, H. 1983. A study of marine oil pollution in outdoor model ecosystems representing a tidal flat (OPEX). Netherlands Organization for Applied Scientific Research, the Hague, Netherlands. Report R83/14, 1–103.

Kuiper, J., de Wilde, P.A.W.J., Wolff, W.J. 1984. Oil pollution experiment (OPEX): I — Fate of an oil mousse and effects on macrofauna in a model ecosystem representing a Wadden Sea tidal mudflat. *In* Persoone, G., Jaspers, E., Clause, C., ed., Ecotoxicological testing for the marine environment: Volume 2. State University of Ghent and Institute for Marine Scientific Research, Bredene, Belgium. 331–359.

Kuiper, J., van het Groenewoud, H., Admiraal, N., Scholten, M., de Wilde, P.A.W.J., van Moorsel, G., Dekker, R., Wolff, W.J., Brouwer, C. 1986. The influence of dispersants on the fate and effects of oil in model tidal flat ecosystems. Final report on Oil Pollution Experiments, with special reference to the use of Finasol OSR5 (OPEX 1984–1986). Netherlands Organization for Applied Science Research, the Hague, Netherlands. Report R86/182, 1–94.

Meuzelaar, H.L.C., Kistemaker, P.G., Eshuis, W., Boerboom, H.A.J. 1977. Automated pyrolysis mass spectrometry; application to the differentiation of micro-organisms. *In* Daly, N.R., ed., Advances in mass spectrometry, Volume 7B. Heyden, London, UK. 1 452–1 456.

Newell, G.E. 1948. A contribution to our knowledge of the life history of *Arenicola marina*. Journal of the Marine Biological Association of the United Kingdom, 27, 554–580.

Reise, K. 1977. Predator exclusion experiments in an intertidal mudflat. Helgolaender Wissenschaftliche Meeresuntersuchungen, 30, 263–271.

Ringelberg, J. 1976. The possibilities of a new kind of micro-ecosystem in the aquatic ecosystem research. Hydrobiological Bulletin, 10, 17–18.

Straaten, L.M.J.U. van 1952. Biogene textures and the formation of shell beds in the Dutch Wadden Sea. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen (B), 55, 500–516.

Vosjan, J.H., Olanczuk-Neyman, K.M. 1977. Vertical distribution of mineralization processes in a tidal sediment. Netherlands Journal of Sea Research, 11, 14–23.

Witte, F., de Wilde, P.A.W.J. 1979. On the ecological relation between *Nereis diversicolor* and juvenile *Arenicola marina*. Netherlands Journal of Sea Research, 13, 394–405.

Bremerhaven Caissons — Experience and Results of Experiments with Dispersed Crude Oil in Intertidal Enclosures

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The Bremerhaven Caissons were designed and constructed to enclose parts of aquatic ecosystems for field experiments, especially on intertidal sand flats and mud flats. The constructions are container-like boxes that are open at the top and bottom. Four walls (2 m high × 5.6 m wide × 2.35 m long) enclose an area of about 13 m^2. A Bremerhaven Caisson can operate in a closed, semiclosed, or flow-through mode. Enclosed are both the benthic and the planktonic systems. Long-term experiments (up to several months) on the benthos are possible independent of the operational mode. For plankton, the experimental period depends on the period the water remains in the caisson. Thus, long-term experiments on this part of the ecosystem are only possible when the water circulation is closed.

In pollution experiments, caissons were used to study the fate and effects of chemically and ultrasonically dispersed crude oil and a dispersant on an intertidal benthic system. In different experiments, oil in concentrations between 2 and 40 mg·L^{-1} was added to the water inundating the caisson interior during the flood tide (semiclosed mode). The fate of the oil in the sediment (penetration, residence time, etc.) and the effects of both kinds of dispersion and the dispersant on microphytobenthos, bacteria, meiofauna, and macrofauna were studied. The results generally show that oil concentrations below 20 mg·L^{-1} have, in most cases, reversible sublethal effects. Higher oil concentrations for a period of several days induced heavy mortality in some groups of the ecosystem, especially in macrofauna species. No difference between chemically and ultrasonically dispersed oil was observed.

In the southeastern part of the North Sea, a spacious intertidal area of about 800 km^2 extends along the Dutch, German, and Danish coasts, forming the Wadden Sea (Fig. 1). As the ground slopes very slightly, this intertidal zone has an average width of about 10 km and a maximum extension of 20 km. The region is characterized by sand flats and mud flats inundated and drained by many tidal channels.

A chain of dune islands and sand barriers forms the seaward border between the Wadden Sea and the subtidal North Sea. This area is of great ecological

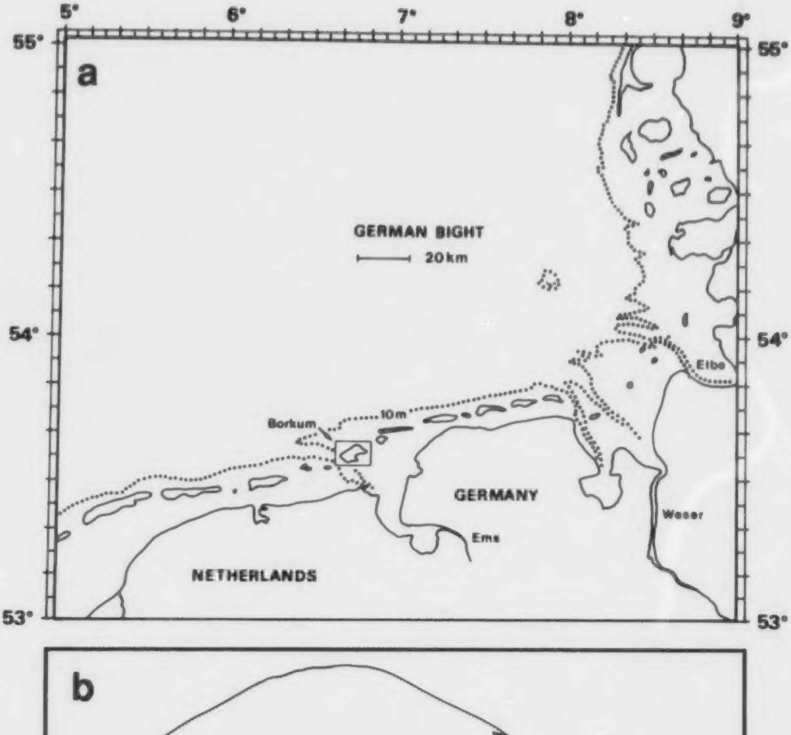

5°
6°
7°
8°
9°
55°
55°
54°
54°
53°
53°
a
GERMAN BIGHT
20 km
Borkum
10m
Elbe
GERMANY
Weser
Ems
NETHERLANDS
b
Island of Borkum
HWL
LWL
HWL = High Water Level
LWL = Low Water Level
● = Experimental Site

Fig. 1. (a) German Bight and (b) intertidal area south of the island of Borkum.

significance. Its high secondary production, based on the rich microphytobenthos of most flats and sedimentation of organic material from the North Sea, provides ample food for a large number of consumers, such as young fish, crabs, and shrimps during the submersion period and birds during the emersion period.

Experimental ecosystem research in the Wadden Sea has usually been carried out in the field by direct manipulation of the bottom or by setting up smaller devices, such as cages, to exclude predators (Reise 1985). In the laboratory, benchtop container systems were used to investigate special questions, such as the reproduction, biology, and life cycles of intertidal species (Farke and Berghuis 1979). The Kiel Plankton Tower and the Hamburg Enclosures (Grice and Reeve 1982) were the first German facilities used to enclose marine plankton communities. They were used during the 1970s in the Baltic Sea and a Norwegian fjord. In the subtidal zone of Kiel Bay, a medium-scale experiment on macrozoobenthic colonization, succession, and secondary production, the "Benthosgarten," was carried out between 1975 and 1979 (Arntz and Rumohr 1982, 1986; Rumohr and Arntz 1982). A first enclosure system in the Wadden Sea was set up in the early 1970s for heavy metals studies (de Wolf et al. 1972). In this system, part of an intertidal mussel bed was palisaded for a summer period and the water inundating the interior in the tidal rhythm was contaminated with copper. At the end of the experiment, the timber construction had to be totally demolished. Further experiments employing this enclosure technique were not carried out.

In 1975, "large indoor tidal mud flat ecosystems" (de Wilde and Kuipers 1977) were established at the Netherlands Institute of Sea Research, Texel. Two mesocosms, each containing Wadden Sea sediment covering an area of 20 m^2 and containing 60 m^3 of seawater, circulating between storage tanks and the sediment boxes in a tidal rhythm, were constructed in an aquarium. Using the "indoor tidal mud flat ecosystems," basic research was carried out in an attempt to understand the Wadden Sea ecosystem. Since 1981, the Dutch have used modifications of these mesocosms, so-called MOTIFs (model tidal flats), to conduct research on the effects of oil and dispersants in the Wadden Sea (Kuiper et al. 1986). The MOTIFs are set up on shore in outdoor basins on the island of Texel. As the sediment structure and the benthic community are disturbed as a result of being transported from the tidal flat and introduced into the mesocosms, the large "indoor tidal mud flat ecosystems" as well as the "MOTIFs" need a longer period of stabilization before experiments begin.

Contrary to these on-shore mesocosms, the basic principle of the Bremerhaven Caissons (Fig. 2) is related to the offshore enclosure used in the Netherlands by de Wolf et al. (1972). In both systems, natural areas of tidal flats are enclosed by walls so that experiments can be carried out inside. An advantage of the Bremerhaven Caissons is that they are mobile. They can be brought into position on a flat within one tidal period and can be moved away at the end of an experiment during another tidal period. Neither operation disturbs the experimental site, so the development of the benthos after the experiments can be followed under natural conditions.

The caissons were used at a time when pollution problems associated with coastal North Sea and the sensitivity of researchers to the vulnerability and uniqueness of the Wadden Sea area were increasing. Because little was known about the fate and effects of pollutants under natural conditions on the Wadden Sea ecosystem, the caissons were constructed to fill the gap between benchtop laboratory

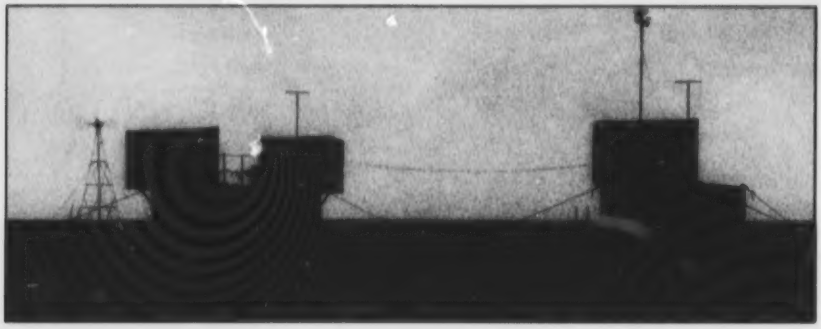


Fig. 2. Bremerhaven caissons on an intertidal sand flat south of the island of Borkum.

experiments and field observations (Parsons 1982). Between 1981 and 1985, 18 long- and short-term experiments on the fate and effects of heavy metals and oil dispersants were carried out in the caissons. Since 1985, the facilities have been used for fundamental research to study interactions between predators and prey, especially juvenile macrofauna, on the tidal flats.

Bremerhaven caisson

Technical description

A Bremerhaven caisson consists of a rectangular container that is open at the top and bottom (for technical details, see Farke et al. (1984)). The walls, made of seawater-proof aluminum, are 5.6 m long (sidewalls), 2.35 m wide (front walls), and 2 m high. About 13 m^2 of a tidal flat are enclosed. Instruments and the electric power supply are contained in two canvas shelters mounted on platforms at the upper outer edge of the front walls. Researchers can find shelter there during their stay on the caissons. A gangway connects the two platforms. Inside the caisson, about 0.5 m above the sediment surface, a movable working bridge allows researchers to sample the sediment and benthos without walking on it. Windows in the sidewalls reduce shading (Fig. 3).

Equipment

Scour protection and mooring on the ground

Protection against scouring is indispensable if caissons are to be operated in the field. Synthetic mats, as used in aquatic engineering, provide effective scour protection. They are fastened inside at the lower edge of the walls and run underneath to the outside so that the caisson rests on them. These mats are 1 m wide and cover the sediment around the caissons. Their outer edges are dug into the ground, providing a smooth transition between the sediment and mat. As an additional safety measure, two layers of densely packed sandbags surround the walls of the caisson (Fig. 2).

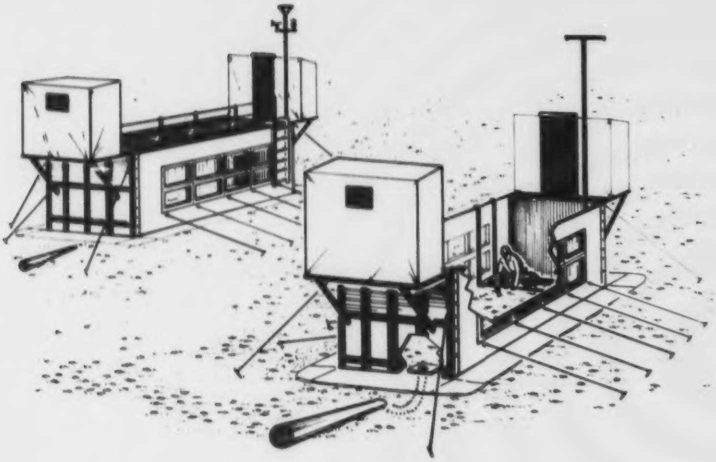


Fig. 3. Caissons on an experimental site (from Farke et al. 1984).

To prevent any movement, each caisson is moored by 16 guy ropes that are fixed to iron pins screwed about 1 m into the ground.

Inundation systems

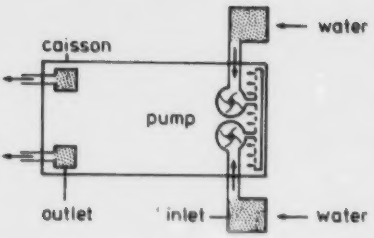
The caissons can be operated in three different ways: as a flow-through system, semiclosed, and closed (Fig. 4). The flow-through mode (Fig. 4a) is used for predator–prey experiments. In this case, water from the flats is continuously pumped into the caisson during the submersion period. The incoming water, which is filtered through 1-mm meshes, is dispersed by a tube system close to one end wall. At the opposite wall, an outlet system equalizes the water surplus from the pumps so that the same water level is maintained inside and outside the caisson.

The semiclosed mode (Fig. 4b) is used for pollution experiments. In this case, one inundation system functions as a water inlet during the flood period and as an outlet during the ebb tide. Within one submersion period, no water exchange between the interior of the caisson and the ambient flat takes place and the contamination can be confined to the water volume inundating the caisson during the flood tide. For long-term pollution experiments, the total water exchange within the tidal rhythm necessitates contaminating the incoming water for each subsequent flood.

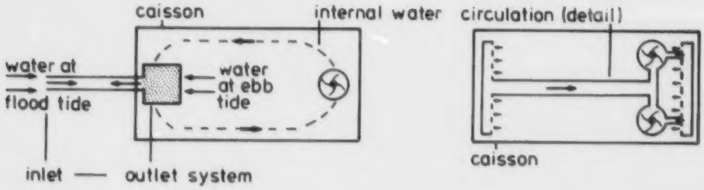
In the semiclosed mode, a water-circulation system must be installed inside the caisson to avoid oxygen depletion in the water close to the bottom. For circulation, the caisson water is pumped to a row of horizontally fixed outlets near one end wall. On the opposite end wall, a similar pipe system functions as an inlet leading the water back to the pumps.

Operating the caisson in a closed mode (Fig. 4c) seems possible but has not yet been tried. In this case, a tank should be installed on a ship anchored near the caisson to store the caisson water during periods of emersion. Inundation and drainage

a. flow-through mode


water
caisson
pump
outlet
inlet
water

b. semi-closed mode


caisson
internal water
circulation (detail)
water at
flood tide
water
at ebb
tide
inlet — outlet system
caisson

c. closed mode

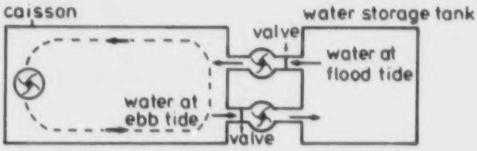

caisson
water storage tank
valve
water at
flood tide
water at
ebb tide
valve

Fig. 4. Operational modes of the caissons.

of the caisson's interior must be regulated by valves and pumps corresponding to the tidal regime on the flat.

Electric power supply

Electric power is mainly used for pumping the water inside the caisson (flow-through mode) and for water circulation (other operational modes). Electricity is also needed for the tide-gauge controlled automatic addition of soluble pollutants (Farke et al. 1984) developed for heavy metal experiments. To diminish risks, only low voltage electricity (12-V direct current) is available on the caisson, so all pumps and other electric facilities must operate under this condition. Eight special batteries for slow discharge, each with a capacity of 60 A-h, are installed. They are recharged by a wind generator and during periods of low wind velocities by a small 12-V generator. A sophisticated electronic unit controls discharging and recharging the batteries and switches off the generator or the consumer in case of overcharging or undercharging. In this way, the batteries and the whole electric-power system are protected from destruction.

Environmental and meteorological data

Various environmental and meteorological parameters — salinity; sediment, water, and air temperature; tide-gauge data; water turbidity; radiation; precipitation; and the direction and speed of the wind — are continuously recorded (every 5 min) and stored on magnetic tape.

Operation of the caisson

At present, the Bremerhaven caisson is the only mobile mesocosm capable of enclosing the benthos. Using two rubber floats attached to the sidewalls, it can be towed to the experimental site when the flat is submerged (Fig. 5). With a draft of only 0.3 m, the caisson can reach even very shallow parts of the Wadden Sea. When the tide recedes, the caisson rests on the flat. After detaching the floats and extending the scour-protection mats, the device is moored to the ground. Exact positioning on an experimental plot is possible within a 0.3-m limit, so that the site can be prepared before the caisson is settled. The caisson can be remobilized by reattaching the floats to the sidewalls, which lift the device from the bottom during the next flood period. Even after standing in one place for 2 months, the floats are sufficiently buoyant to lift the caisson with the attached scour-protection mats. The caisson can then be moved to another site nearby within one submersion period.

Field experience

Between 1980, when the devices were constructed and the first field trial took place, and 1987, the Bremerhaven caissons have proven their practicability for ecological research in 21 field experiments carried out during the period from May to September each year in different parts of the German Wadden Sea. During these periods, the caissons were used for 9 short-term (1 week) and 12 long-term (1–2 months) experiments on the fate and effects of heavy metals (Prosi et al. 1983; Rehm and Schulz-Baldes 1983; Schulz-Baldes et al. 1983; Farke et al. 1985a,b), oil and dispersants, and benzpyrene and to study predator–prey interactions in the intertidal ecosystem.

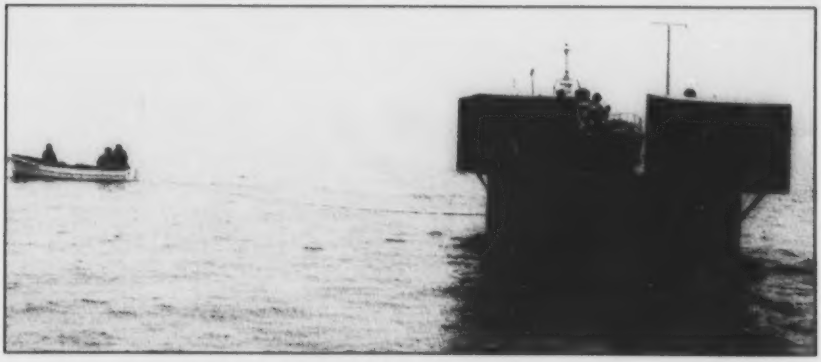


Fig. 5. Caisson being towed to an experimental site.

During all of these operations and the long periods in the field, the caissons have been exposed to manifold weather conditions. Although experiments usually start after the stormy period in spring and normally end before the autumn gales occur, the caissons have to withstand heavy weather conditions nearly every year. Thunderstorms with violent gusts as well as storms up to 9–10 Beaufort wind speed (75–100 km·h^{-1}) were endured for one or two tidal periods without having any major affect on the experiments. Only extreme high tidal water levels, which in the German Bight result from strong northwesterly winds, and storms may cause problems. A few times, water levels as high as the upper edge of the caisson and waves endangered the platforms and canvas shelters, but the scour-protection mats, sandbags, and moorings prevented sediment erosion around the walls and any dislocation of the caisson. Although no operation failed, such events mark the limits of caisson experiments. As the devices are only 2 m high, the mean water depth at high tide at the experimental site should not exceed 0.8–1 m because storm tides with water levels about 1 m above mean high tide level sometimes occur in the German Bight even during summer. Thus, caisson experiments in deeper parts of the Wadden Sea, near the low-water mark, could only be carried out under stable weather conditions and special security measures.

Effects resulting from enclosure by caissons have been mentioned in Farke et al. (1984) and current experiments confirm former appraisals. Reductions of light by the caisson walls results in lower primary production of microphytobenthos at the beginning of an experiment, but this seems to be normalized after a period of adaptation. Generally, primary production of the microphytobenthos in the Wadden Sea is unstable, with marked differences within a few days. Inside the caisson, the bottom temperature is only 1–2°C higher than outside the caisson, even on hot sunny days, and extra sedimentation is limited to an extent that is not obvious because of sediment reworking by bioturbation activities.

Operating the caisson in the semiclosed mode without water circulation results in a marked reduction of oxygen in the enclosed water body, which may lead to mortality of benthic macrofauna. The system of water circulation prevents such effects, and oxygen measurements of near-bottom water show an oxygen decrease of about 10% only during the ebb period when the influx of seawater from the flats is stopped.

Measurements of algal chlorophyll in bivalves can be used as an indication for the feeding activity of these animals (Farke and Gunther 1984). A comparison of the chlorophyll content in *Cerastoderma edule* from the flats and from a caisson during a period of 9 days (Fig. 6) shows no major differences. Species composition and abundance of macrozoobenthos between the control caisson and the flats did not differ markedly, even after periods longer than 1 month.

Experiments with oil and dispersants

The Wadden Sea ecosystem is very sensitive to oil pollution. In addition to acute toxic effects after an oil spill, sandy and muddy sediments will incorporate stranded oil and become a source of chronic oil pollution for many years. In spite of this, highly frequented shipping routes exist only a few kilometres away from the Wadden Sea. Difficult navigational conditions and often stormy or misty weather enhance the risks of collisions or tankers becoming stranded and leaking oil.

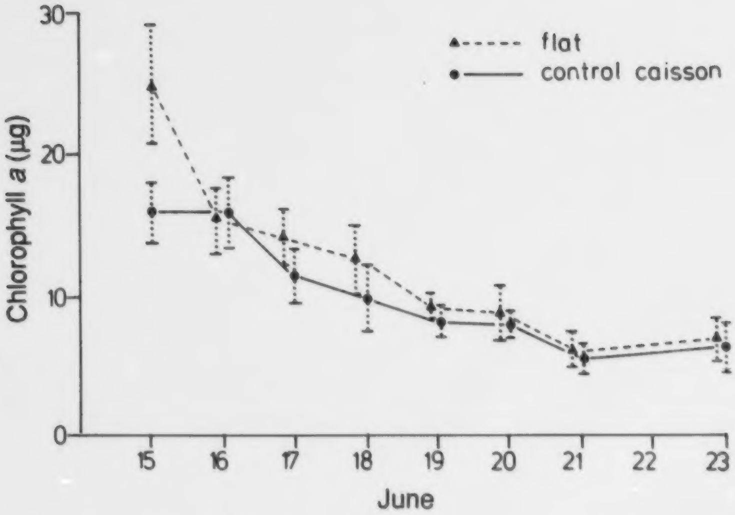


Fig. 6. Feeding activity of *Cerastoderma edule* from an uncontaminated caisson and from the ambient flat. The amount of algal chlorophyll *a* ingested by the bivalves is indicated.

In addition to mechanical cleanup, which is favoured in Germany, chemical dispersion of oil is now being discussed in light of the modern dispersants of low toxicity that have been developed. The Bremerhaven Caissons have allowed researchers to contaminate a defined water body with dispersed oil and a dispersant. In different experiments, chemically and ultrasonically dispersed oil and a pure dispersant were added to the caisson water to study and compare the fate and effects of the contaminants in the benthic ecosystem. The experiments were conducted to provide information about the ecological consequences on tidal flats when chemically dispersed oil enters the Wadden Sea with the incoming flood. In the case of a near-shore oil spill, the results may help in deciding whether dispersants should be used to combat the oil.

During the period 1982–1984, nine experiments involving various concentrations of dispersant and dispersed crude oil were carried out (Table 1). The oil was dispersed either chemically or ultrasonically. In all experiments, the contaminant was added over several succeeding tides to the water inundating the caisson during the flood period. The results of the 1982–1983 experiments have been published (Farke et al. 1985a,b) and a detailed report including 1984 data is in preparation. Thus, only the main results are reported here.

Accumulation of oil in the sediment

In all experiments, even at the highest concentration of oil, 40 mg·L^{-1} of seawater, the dispersed oil accumulated only at the surface of the sediment, with little penetration into deeper layers. As a consequence, the period of oil contamination of the benthic system was short, because after refloating the caissons the natural water movement (waves and currents) and biological degradation eliminated the oil from the surface sediment within 1–3 months. Only in the experiment with oil at

Table 1. Oil dispersant experiments with Bremerhaven Caissons, 1982–1984.

Experiment	Contaminant	Concentration (ppm)	Contamination period (d)
82–1	AL/Fi	4	3
82–2	AL	2	3
82–3	Fi	0.4	3
83–1	Fi	1	6
83–2	AL/Fi	10	6
83–3	AL	8	6
84–1	St/Fi	40	6
84–2	St/Co	20	6
84–3	St/Fi	20	6

Notes: In experiments with oil and oil-dispersant mixtures, the values refer to the oil concentrations actually measured in the caisson water during high tide (mean) of each contamination period. In experiments with pure dispersant, nominal concentrations have been calculated.

Abbreviations: AL, Arabian light crude oil; St, Staffjord crude oil; Fi, Finasol OSR5; and Co, Corexit 9527.

40 mg·L^{-1} of seawater were slightly increased oil concentrations measured even 90 days after the contamination period. However, compared with the real-world situation after an oil spill, where large areas are polluted, only an area of 13 m^2 was contaminated during these experiments. Thus, elimination of oil from the entire Wadden Sea region would be much slower. Because no deep sediment layers were seriously contaminated, a state of chronic pollution may be avoided by dispersing the oil.

Biological investigations

Various biological disciplines were involved in the experiments. Investigations were carried out on microphytobenthos, bacteria, meiofauna, and macrofauna; thus, the response of important parts of the Wadden Sea ecosystem to oil and the dispersants was studied. Generally, no biological effects were observed when adding the dispersant alone, and different reactions could not be found between the chemical and ultrasonic dispersion of the oil. In all of the experiments, the measured effects depended on the concentrations of the dispersed oil in the water and the time of exposure.

The gross photosynthetic rate of microbenthic algae (mostly diatoms) calculated from oxygen production and consumption measurements was used as an indicator of algal activity. At oil concentrations of 2–4 mg·L^{-1} of seawater, the gross photosynthetic rate increased, but at 10 mg·L^{-1}, an increase in algal activity was partially inhibited compared with the control caisson. At 40 mg·L^{-1}, no algal activity could be measured.

Microbiological investigations showed no toxic effects of the dispersions on oil-degrading bacteria even at the highest concentrations. Compared with the controls, their numbers increased by a factor of 10–100. An adaptation of the bacterial populations occurred within a few days. Biological oxygen demand (BOD) measurements showed that biological degradation of the oil plays a role in the elimination processes.

For nematodes, the most abundant meiofaunal group, the number of species, abundance, and diversity were studied. Generally, nematodes seem to be less affected by oil, and the parameters mentioned were not very helpful for indicating effects, especially when the data referred to the nematode group as a whole. Shifting dominances could be found only at higher oil concentrations.

Clear effects at all levels of contamination with oil were observed in macrobenthic animals. Oil concentrations up to 10 mg·L^{-1} of seawater caused mainly sublethal effects, such as a reduction in the feeding activities of bivalves and polychaetes (Farke and Guenther 1984; Farke et al. 1985a,b). Only a few species showed decreased abundances. At 20 mg·L^{-1}, *Cerastoderma edule* and *Mytilus edulis* stopped digesting algal chlorophyll, and some polychaetes and bivalves appeared at the sediment surface. However, no mass mortality occurred, and most of the animals dug again when the contaminant were removed.

Doubling the amount of oil to 40 mg·L^{-1} of seawater caused severe effects on macrobenthic animals (Table 2). After the second contamination, all *C. edule* appeared on the sediment, but regeneration experiments showed that all of these animals were able to dig again after being kept for 1 day in clean seawater. After four contaminations, only 35% of the cockles could regenerate; after eight contaminations, none of the cockles could regenerate. Another bivalve, *Macoma balthica*, appeared on the sediment at the end of the contamination period, but the percentage that could regenerate was low. Higher numbers of the polychaete *Arenicola marina* were found on the sediment after the second contamination. After six additions of oil, its regenerating ability dropped to 57% and none were able to dig after eight contaminations. *Nereis diversicolor*, another polychaete, was more sensitive. Only 50% regenerated after two oil additions.

About 90% of the macrofauna was killed during this experiment. Of the 500

Table 2. Effects of Staffjord crude oil (40 mg·L^{-1} of seawater) dispersed by Finasol OSR5 on four endobenthic macrofauna species.

	Contaminations						
	1	2	4	6	8	10	12
Cerastoderma edule							
No. of individuals[a]	250	676	658	606	676	588	—
Successful regeneration (%)	—	100	35	13	0	—	—
Macoma balthica							
No. of individuals	0	0	0	0	14	29	1 102
Successful regeneration (%)	—	—	—	—	30	20	—
Arenicola marina							
No. of individuals	0	2	100	180	208	177	—
Successful regeneration (%)	—	100	100	57	0	—	—
Nereis diversicolor							
No. of individuals	Many	Many	Many	Many	Many	—	—
Successful regeneration (%)	100	50	—	20	0	—	—

[a] Number of individuals appearing at the sediment surface.

lugworms living in the caisson area at the beginning of the contamination period, only 25 survived. Other species, such as *Nereis diversicolor* and *Cerastoderma edule*, were totally absent after the experiment. Recolonization started soon, however, and after about 6 months the abundance of most species at the experimental site was comparable to the uncontaminated flats. Again, the surrounding unaffected areas provided a large reservoir for resettlement. In the case of a real oil spill, however, regeneration of an entire intertidal region would be expected to be much slower.

Conclusions

As noted by Giesy and Odum (1980), short-term single-species laboratory tests are, in most cases, insensitive to important interactions or compensatory reactions between the organism and its abiotic and biotic environments. As the complexity of natural systems can be realized to some extent in micro- or mesocosms, they may function as operational bridges between simple laboratory tests and complex field situations.

On the other hand, unavoidable impacts, spatial and temporal limitations, and changes in the abiotic and biotic environments affect the comparability of micro- and mesocosms with natural ecosystems and produce different kinds of stress (Pilson and Nixon 1980). In pollution studies, the stress caused by the experimental system itself and additional pollutant stress may have synergistic effects or they may alleviate each other. Thus, enclosures of natural ecosystems, the more complex they are, may provide decreasing possibilities to isolate cause and effect (Steele 1979).

Inherent impacts of the system differ from one experimental setup to another and depend on the type of enclosure. As shown by the response to copper pollution of two large plankton enclosures at Saanich Inlet and Loch Ewe, pelagic ecosystems may be critical variables themselves, limiting their use in pollution research (Steele 1979). Benthic organisms, especially macrofauna, seem to be better pollution indicators than plankton (Banse 1982). With a mainly sedentary lifestyle and longer life spans, macrobenthos might integrate environmental effects more significantly than planktonic organisms. The Bremerhaven Caissons are mainly designed for benthos research, and in the pollution experiments only this part of the intertidal ecosystem was studied. Plankton and seston, which are transported into the caissons twice a day, develop outside under natural conditions. Thus, caisson- or pollutant-specific plankton do not establish, diminishing the risk of artifacts. Before the experiment, the sediment as well as the benthos can develop under natural conditions; thus, the historical component of the ecosystem is kept intact in caisson experiments. After refloating the caisson, the experimental area is returned to natural conditions again; thus, loss and degradation of pollutants and regeneration of the benthic system can be studied.

At present, two Bremerhaven Caissons have been constructed: one is used for experimental manipulations; the other is used as a control system. A team of about 10 people can install the caissons on the flat within one emersion period and then operate the two devices in the field. To adequately replicate experiments would double or triple the work and costs involved, thus going beyond practical limits for most tidal areas and most research institutes.

To avoid pseudoreplication (Hurlbed 1984), inferential statistics cannot be used to validate the results of caisson experiments. On the other hand, the oil–dispersant studies show that repeated experiments on the same subject, even with a slightly different experimental design, such as increasing oil concentrations, allow conclusions to be drawn on the behaviour, fate, and a great variety of effects of such substances in the natural environment.

Arntz, W.E., Rumohr, H. 1982. An experimental study of macrobenthic colonization and succession, and the importance of seasonal variation in temperate latitudes. Journal of Experimental Marine Biology and Ecology, 64, 17–45.

______ 1986. Fluctuations of benthic macrofauna during succession and in an established community. Meeresforschung, 31, 97–114.

Banse, K. 1982. Experimental marine mesocosms in historical perspective. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 10–24.

de Wilde, P.A.W.J., Kuipers, B.R. 1977. A large indoor tidal mudflat ecosystem. Helgolaender Wissenschaftliche Meeresuntersuchungen, 30, 334–342.

de Wolf, P., Kock, W.C., Stam, A. 1972. Field experiments on the influence of copper and mercury in a natural mussel bed. Netherlands Organization for Applied Science Research, Niews, 27, 497–504.

Farke, H., Berghuis, E.M. 1979. Spawning, larval development and immigration behaviour of *Arenicola marina* in the laboratory. Netherlands Journal of Sea Research, 13, 512–528.

Farke, H., Guenther, C.-P. 1984. Effects of oil and a dispersant on intertidal macrofauna in field experiments with Bremerhaven caissons and in the laboratory. *In* Persoone, G., Jaspers, E., Claus, C., ed., Ecotoxicological testing for the marine environment (volume 2). State University of Ghent, Institute for Marine Scientific Research, Bredene, Belgium. 219–235.

Farke, H., Schulz-Baldes, M., Ohm, K., Gerlach, S.A. 1984. Bremerhaven caisson for intertidal field studies. Marine Ecology Progress Series, 16, 193–197.

Farke, H., Blome, D., Theobald, N., Wonneberger, K. 1985a. Field experiments with dispersed oil and a dispersant in an intertidal ecosystem: fate and biological effects. *In* Proceedings of the 1985 Oil Spill Conference. American Petroleum Institute, Washington, DC, USA. 515-520.

Farke, H., Wonneberger, K., Gunkel, W., Dahlmann, G. 1985b. Effects of oil and a dispersant on intertidal organisms in field experiments with a mesocosm, the Bremerhaven caisson. Marine Environmental Research, 15(2), 97–114.

Giesy, J.P., Jr., Odum, E.P. 1980. Microcosmology: introductory comments. *In* Giesy, J.P., Jr., ed., Microcosms in ecological research. National Technical Information Service, Springfield, VA, USA. Department of Environment Symposium Series 52, CONF-781 101, 1–13.

Grice, G.D., Reeve, M.R. 1982. Introduction and description of experimental ecosystems. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 1–9.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs, 54, 187–211.

Kuiper, J., Groenewoud, H. van het, Admiraal, N., Scholten, M., de Wilde, P.A.W.J., Moorsel, G. van, Dekker, R., Wolff, W.J., Brouwer, C. 1986. The influence of dispersants on the fate and effects of oil in model tidal flat ecosystems — Final report on Oil Pollution Experiments, with special reference to the use of Finasol OSR5 (OPEX 1984–1986). Netherlands Organization for Applied Science Research, the Hague, Netherlands. Report R86/182, 94 pp.

Parsons, J.R. 1982. The future of controlled ecosystem enclosure experiments. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 411-418.

Pilson, M.E.O., Nixon, S.W. 1980. Marine microcosms in ecological research. *In* Giesy, J.P., Jr., ed., Microcosms in ecological research. National Technical Information Service, Springfield, VA, USA. Department of Environment Symposium Series 52, CONF-781 101, 724–741.

Prosi, F., Loring, D.M., Mueller, G. 1983. Cadmium cycling between water, sediment and biota in an artificially contaminated mudflat of the North Sea. *In* International Conference on Heavy Metals in the Environment held in Heidelberg, September 1983. CEP Consultants, Edinburgh, UK. Volume 2, 819–822.

Rehm, E., Schulz-Baldes, M. 1983. The absorption of lead in intertidal sediments: in situ experiments with the Bremerhaven caisson. *In* International Conference on Heavy Metals in the Environment held in Heidelberg, September 1983. CEP Consultants, Edinburgh, UK. Volume 2, 968–971.

Reise, K. 1985. Tidal flat ecology: an experimental approach to species interactions. *In* Billings, W.D., Golley, F., Lange, O.L., Olson, J.S., Remmert, H., ed., Ecological studies, 54. Springer-Verlag, New York, NY, USA.

Rumohr, H., Arntz, W.E. 1982. The "Benthosgarten" — a new approach for the study of soft-bottom communities. Meeresforschung, 29, 225–238.

Schulz-Baldes, M., Rehm, E., Farke, H. 1983. Field experiments on the fate of lead and chromium in an intertidal benthic mesocosm: the Bremerhaven caisson. Marine Biology, 75, 307–318.

Steele, J.H. 1979. The uses of experimental ecosystems. Philosophical Transactions of the Royal Society, London (Series B, Biological Sciences), 286, 583–595.

A Subtidal Soft-Sediment Mesocosm

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A subtidal soft-sediment benthic mesocosm is described. Benthic sediments imported into the mesocosm showed variations in sediment chemistry within the range of those found in the field. Dominant faunal groups of species remained similar in composition to those in the field over a period of 6 months. Recruitment was, however, lower within the mesocosm than in the field. A range of different experiments have been conducted that cannot be carried out adequately in the field. Detailed studies of bioturbation are especially important.

Most mesocosms in use today enclose the water column (see Grice and Reeve (1982) for a review). This is not surprising considering that the idea of conducting experiments on a large scale in the sea was first suggested by John Strickland, who was primarily interested in the water column. A few mesocosms have been designed to integrate the water column and the sediment, notably the MERL system (Pilson et al. 1977) and the Kiel Bay facility (Bodungen et al. 1976). In the latter two systems, the emphasis was on interactions between the water column and the sediment, and the sediment could only be sampled through the water column.

Two intertidal sediment mesocosms have been designed, the Texel facility in Holland (de Wilde, this volume) and the German Caisson systems (Farke et al., this volume). In these mesocosms, the primary focus of research has been the benthos and processes affecting the benthos. To date, no facilities are concerned with subtidal sedimentary processes.

Subtidal areas, particularly of the continental shelves, cover a large area and are of great economic importance for fisheries and for mineral extraction; however, they are increasingly being subjected to large amounts of pollution. Thus, understanding the processes that occur within subtidal sediment or at the sediment–water interface is of vital importance.

A subtidal sediment mesocosm facility was designed and built on the Oslofjord, Norway, with financial assistance from British Petroleum Norge A/S. The facility is owned by the Norwegian Institute of Water Research (NIVA), and is operated jointly with the University of Oslo. In this paper, the performance of the facility, the types of experiments carried out, and the future use of such a facility are considered.

Design of a subtidal sediment mesocosm

Conducting field experiments on muddy subtidal sediments is exceedingly difficult. Scuba diving has depth limitations and both Scuba diving and the use of submersible vehicles disturb the surface layers of sediments, particularly the flocculent organic material. It is extremely important that the structure of the sediment be maintained if experimental work is to be carried out. Thus, one of the primary design criteria involved taking large intact samples of the seabed while retaining the surface flocculent material. This criterion dictated the use of a large box-core sampler (i.e., a modified USNEL box corer built by Adolf Wuttke, Hamburg, capable of taking a 0.25-m^2 sample). In the MERL system, sediment samples extracted from the box corer are placed adjacent to each other on a circular tray. This manipulation of the sediment probably disturbs the surface layer. Therefore, the samples used in this study took intact 0.25-m^2 samples (taken with a core liner within the sampling box) into the system and experimented on large numbers of replicate boxes.

The design of the mesocosm was, in part, dictated by its location at a redundant trout farm. Here, two of the original basins (4.9 m × 21.5 m and 4.6–5.0 m × 21.5 m) were converted into an indoor facility and each basin was subdivided into three, giving six experimental sections. The basins were coated with epoxy resin.

Temperature within the system was maintained close to that in the fjord by pumping water from a depth of 42 m with an impeller pump. Throughout a complete year, the temperature ranged from 5 to 11°C; in the summer, the temperature within the system was 1–2°C above that of the intake water temperature. Salinity remained at the same value as that at the intake.

It was thought that the pumping system used would affect larval recruitment as many of the larvae were expected to be damaged upon passing through the pump and pipeline. A quantitative study was conducted to assess the reduction in recruitment. Water flow over the sediment surface is a critical factor influencing a wide range of benthic processes, such as recruitment, nutrient flux, respiration, and degradation of organic matter. Hourly measurements taken 2 m above the seabed at a depth of 30 m over an entire year revealed a surprisingly large range in current readings (0–10 $cm \cdot s^{-1}$) for an area with small (20 cm) tides. The highest velocities occurred between October and December, coincident with autumn storms, but in April and May a daily mean of 1.5 $cm \cdot s^{-1}$ was observed. It was clearly not realistic to mimic the natural water flow regime over the sediment.

Water flow over the sediment surface, therefore, was designed to remain constant at 1 $cm \cdot s^{-1}$. Inflow to each section occurs through a horizontal pipe, with 2.5-mm diameter holes placed 5 cm apart, 60 cm above the basin bottom. Water flows in a laminar fashion across the basin to a horizontal outflow pipe with 4.5-mm diameter holes placed 5 cm apart. The height of the outflow pipe can be adjusted from 0 to 1.7 m. Figure 1 shows a cross section of the basin. Water flow into each basin is regulated by adjustable valves, giving a flow rate of 4 $m^3 \cdot h^{-1}$ into each basin. As the lower water flow rate, compared with actual field conditions, probably reduced sedimentation within the system, this was assessed quantitatively.

The community used in the initial experiments was obtained from Bjørnhodebukta, Oslofjord, where extensive data on benthic communities were

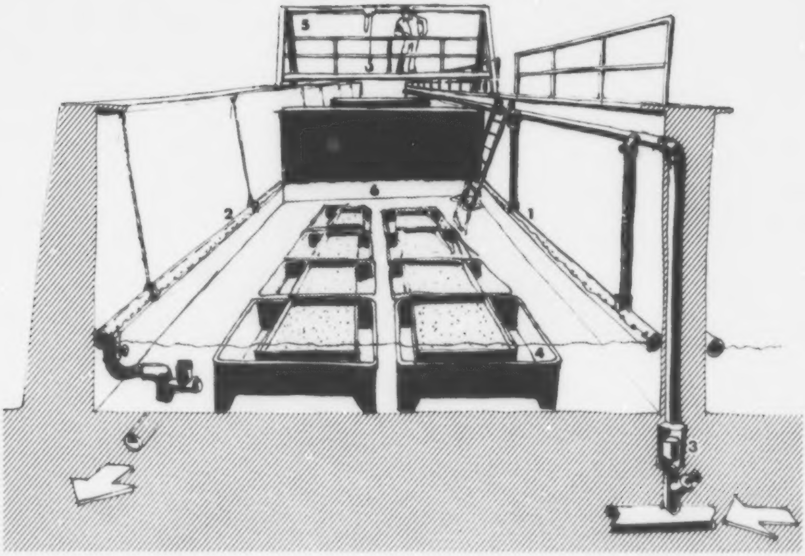

Fig. 1. Schematic of one of the two mesocosm basins. Legend: 1, inflow; 2, outflow; 3, flow meter; 4, box-corer liner with sediment; 5, movable bridge for transporting boxes; and 6, partition dividing basin into three sections (from Berge et al. 1986).

available (Valderhaug and Gray 1984). Thus, the fauna transported to the mesocosm could be compared with that at the field site. As the field sampling station was at a depth of 32 m, it was possible, by Scuba diving, to conduct reciprocal experiments in the field and in the mesocosm.

The Bjørnhodebukta site contains many amphipod species. Most of these undergo diurnal migrations. It was expected that these and other mobile species imported into the mesocosm would decrease in numbers with time because, once they had emerged from the sediment into the water column, they would probably not find their way back into the same or any other box because the sediment surface area was only a fraction of the total area of the basin.

By having a large number of replicate box-core samples, statistically reliable experiments could be run. However, the problem of pseudoreplication (Hurlbert 1984) in mesocosm experiments is acute in this and most other mesocosm systems. Pseudoreplication occurs when, as in this case, individual basins are used with one acting as a control and three being used to study the effects of increasing concentrations of a treatment, with three or more replicate box-corer samples being placed within each treatment. As each treatment is not replicated and the replicates are only taking place within treatments, no true replication is present. Ideally, one should use two complete basins as replicate controls and a similar number for treatments, but the logistical requirements of such a system are formidable. Experience from this study suggests that if the control and treatments are designed to give a dose–response relationship, the response usually reveals a sufficiently clear pattern that the costs, in terms of time and money, of complying with the statistical requirements necessary to avoid pseudoreplication are not justified.

In this study, controls run in two separate basins did not differ significantly. Thus, although all experiments should be carried out in separate basins to avoid pseudoreplication, the costs do not justify this approach.

The advantage of the design used in this study is that one can walk around the sides of the boxes and collect samples and conduct experiments on a small scale. In particular, the bioturbation effects of individual species can be studied quantitatively without disturbance and can be replicated over time. The characteristic bioturbation features of key organisms are now known and their effects on important sedimentary processes can be assessed.

System performance

Two important questions must be addressed in assessing how well a mesocosm system performs in comparison with the natural field situation:

- Over what time periods are the chemical profiles maintained, and
- Over what time periods do organisms survive in similar densities to those found under natural field conditions?

Figure 2 presents data on sediment chemistry from an experiment in which one core was sampled from each of three replicate boxes 0, 7, 11, 13, 17, and 23 weeks after transplantation to the mesocosm. The results show that below the top 2 cm, pS_t, Eh, alkalinity, and ammonium were intermediate between the two field samples taken in February and June. The water content was lower in the mesocosm probably due to sediment compaction during transport. In the top 1 cm of sediment, alkalinity, ammonium, and phosphate were higher in the mesocosm than in the field, probably due to alterations in processes controlling steady-state concentrations during transport (Berge et al. 1986).

Over 23 weeks (Fig. 3), alkalinity, silicate, ammonium, and phosphate decreased, presumably because of a reduction in the input of organic material to the mesocosm. The sedimentation rate measured within the system ($0.017\ g\ C \cdot m^{-2} \cdot d^{-1}$) was an order of magnitude lower than in the field, as expected.

Therefore, it was concluded that sediment can be transported to the system with only minor effects, such as sediment compaction, while the main features of sediment stratification and pore-water properties are maintained. The decrease in organic material was expected and it was the experimental manipulation of the quality and quantity of this material that was planned as a key area of research.

To assess survival of the macrofauna, four box-corer samples were taken at Bjornhodebukta, two were sieved directly and two were placed in the mesocosm for 7 months before the fauna were extracted. Comparison of the macrofauna (Fig. 4) indicates that the three dominant species (*Philomedes brenda*, *Sosane gracilis*, and *Nuculoma tenuis*) maintained their abundances over 7 months, whereas some, *Ophiura affinis*, Amphipoda, *Heteromastus filiformis*, and *Priospio malmgreni*, were less abundant in the mesocosm. All of the less-abundant species are mobile and presumably escape from the boxes. More importantly, no laboratory weeds occurred in the mesocosm section, such as the polychaete *Capitella capitala*, which is abundant in Oslofjord (Berge et al. 1985). In contrast, the tube-building

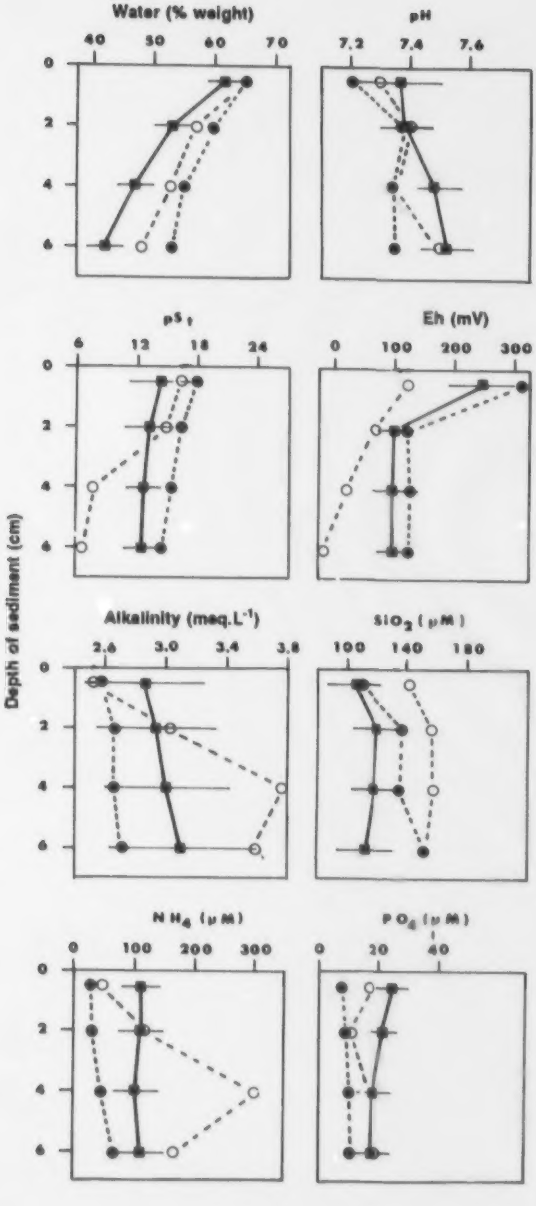

Fig. 2. Comparison of chemical properties of sediment in the field (Bjørnhodebukta) on 7 February (closed circle) and 20 June 1985 (open circle) with mean values from the mesocosm, 11 April – 23 September 1985 (square). $pS_t = -\log$ of total H_2S concentration. Horizontal line = ±1 standard deviation (from Berge et al. 1986).

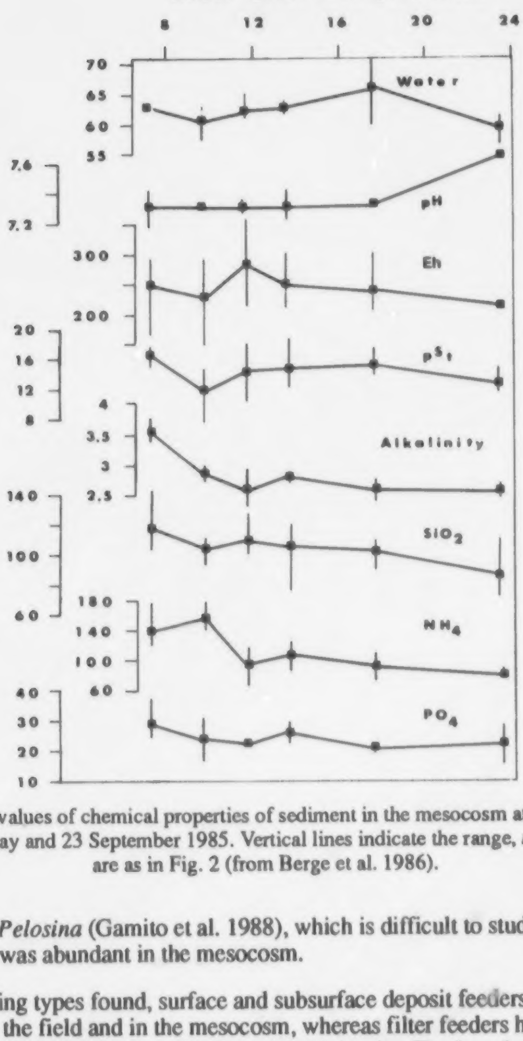

Fig. 3. Mean values of chemical properties of sediment in the mesocosm at 0–1 cm depth between 31 May and 23 September 1985. Vertical lines indicate the range, $n = 3$, and units are as in Fig. 2 (from Berge et al. 1986).

foraminiferan *Pelosina* (Gamito et al. 1988), which is difficult to study alive in field samples, was abundant in the mesocosm.

Of the feeding types found, surface and subsurface deposit feeders had identical abundances in the field and in the mesocosm, whereas filter feeders had only 55% of field density and carnivores only 43% of field density. The low density of filter feeders is undoubtedly due to low amounts of sedimentation compared with that occurring in the field.

As expected, recruitment was low within the mesocosm, with only 45% of the species and only 8.6% of the number of individuals recruited in the mesocosm compared with those recruited in the field. This feature can be used to advantage because experiments can be conducted by adding known larvae to the sediment and measuring quantitative effects on properties of the sediment and on other organisms.

Based upon the results, there seems to be little doubt that the subtidal mesocosm

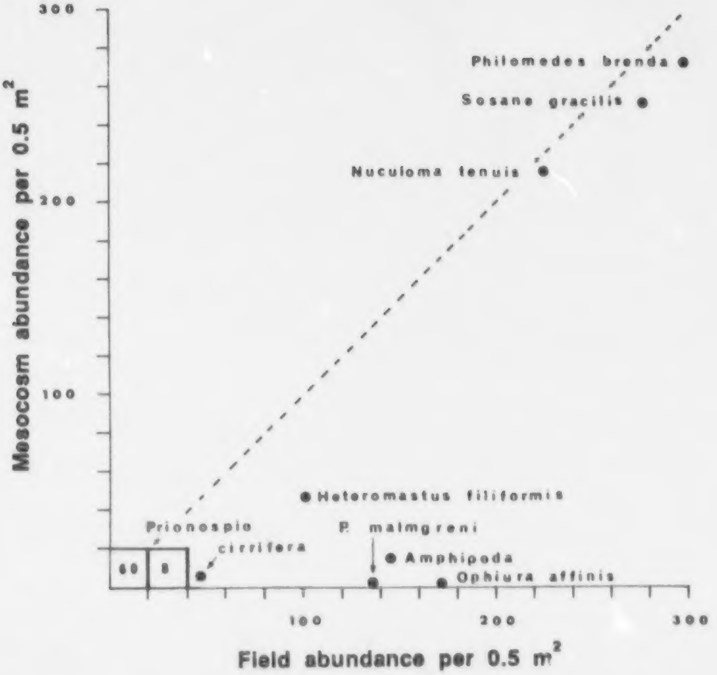

Fig. 4. Comparison of faunal abundance between the field and the mesocosm. The numbers within boxes indicate the number of species found at corresponding densities. The broken line represents perfect similarity (from Gray 1987).

system can maintain the key chemical and biological features that occur in the field over periods up to 6 months. These findings enabled a series of experiments to be completed that could not be carried out in the field or on a laboratory-scale basis.

Research carried since the system was established has looked at the following:

- Basic studies on the biology of sediment-living fauna (Berge et al. 1986);
- Studies on the ambulatory bryozoan *Monobryozoan kinicola* (Berge et al. 1985);
- Effects of polychaete bioturbation on meiofauna (Warwick et al. 1986);
- Effects of organic enrichment on meiofauna (Gee et al. 1985);
- Simulation of sedimentation of the primary bloom to the sediment;
- Addition of fish-farm waste to sediment;
- Experiments on preventing metal release from sediments;
- Spatial distribution of a tube-building foraminiferan (Gamito et al. 1988);
- Maintenance of a community from 200-m depth over 6 months;

- Workshop on the effects of oil and copper on sedimentary systems (Bayne et al. 1988);
- Chemosynthesis in the bivalve *Thyasira* ; and
- Use of planktonic biomarkers for tracing organic material in sediments.

The scale of bioturbation effects by a single tube-building polychaete on the meiofauna (Warwick et al. 1986) has been a significant finding. This work shows that the community structure of the meiofauna was controlled, over a scale of only a few centimetres, by the feeding activities of the polychaete. Clearly, chemical properties of the sediment will also be affected. Arriving at an understanding of sedimentary processes, therefore, must be related to the position of and on scales appropriate to the major bioturbating organism.

Another important study (in preparation) is from the Plymouth Marine Laboratory on chemosynthetic processes in a sediment-living bivalve. The organisms thrived for at least 3 months and manipulations of densities were carried out and important information was obtained through the use of radiotracers on the role that *Thyasira* plays in the flux of sulfur within the sediment.

The fact that communities can be kept for long periods in an almost undisturbed condition, even from 200-m depth, suggests that it will now be possible, to simulate a range of poorly understood processes. One central area of research is the fate of sedimenting organic matter, primarily from the spring plankton bloom. Little is known of the effects of the varying quality and quantity of sedimenting material on the benthos and of degradation processes and how these processes relate to nutrient fluxes to and from the sediment. In the mesocosm, experiments can be carried out on natural communities with careful control of the added material and the bioturbating species.

Following from such research, one should arrive at a better understanding of the processes leading to eutrophication and oxygen deficiency of the bottom water, which are becoming increasingly important pollution problems internationally. Collaboration on this and any of the other topics currently under investigation would be welcomed.

Berge, J.A., Leinaas, H.P., Sandoy, K. 1985. The solitary bryozoan, *Monobryozoon limicola* Frunzen (Ctenostomata), a comparison of mesocosm and field samples from Oslofjorden, Norway. Sarsia, 70, 91–94.

Berge, J.A., Schaaning, M., Bakke, T., Sandoy, K.A., Skeie, G.M., Ambrose, W.G., Jr. 1986. A soft-bottom sublittoral mesocosm by the Oslofjord: description, performance and example of application. Ophelia, 26, 37–54.

Bodungen, B.V., von Brockel, K., von Smetacek, V., Zeitschel, B. 1976. The plankton tower: structure to study water/sediment interactions in enclosed water columns. Marine Biology, 34, 369–372.

Gamito, S.L., Berge, J.A., Gray, J.S. 1988. The spatial distribution pattern of the foraminiferan *Pelosina* cf. *arborescens pearcey* in a mesocosm. Sarsia, 73(1), 33–38.

Gee, J.M., Warwick, R.M., Schaaning, M., Berge, J.A., Ambrose, W.G., Jr. 1985. Effects of organic enrichment on meiofaunal abundance and community structure in sublittoral soft sediments. Journal of Experimental Marine Biology and Ecology, 91, 247–282.

Gray, J.S. 1987. Oil pollution studies of the Solbergstrand mesocosm. Philosophical Transactions of the Royal Society, London (Series B, Biological Sciences), 316, 651–654.

Grice, G.D., Reeve, M.R. 1982. Introduction and description of experimental ecosystems. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 1–9.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs, 84, 187–211.

Pilson, M.E.D., Vargo, G.A., Gearing, P., Gearing, J.N. 1977. The Marine Ecosystem Research Laboratory: a facility for investigation of effects and fate of pollutants. *In* Proceedings of the 2nd National Conference on the Interagency Energy–Environmental R&D Program. US Environmental Protection Agency, Washington, DC, USA. 513–516.

Valderhaug, V.A., Gray, J.S. 1984. Stable macrofauna community structure despite fluctuating food supply in subtidal soft sediments of Oslofjord, Norway. Marine Biology, 82, 307–322.

Warwick, R.M., Gee, J.M., Berge, J.A., Ambrose, W.G. 1986. Effects of feeding activity of the polychaete *Streblosoma bairdii* (Malmgren) on meiofaunal abundance and community structure. Sarsia, 71, 11–16.

Enclosed Plankton Ecosystems in Harbours, Fjords, and the North Sea — Release and Uptake of Dissolved Organic Substances

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Enclosure experiments employing seamless plastic foil tubes 1 m in diameter were performed at sheltered sites (harbours and fjords) and within a drifting float in the North Sea. Release and uptake of dissolved organic matter (DOM), carbohydrates, and amino acids were investigated in natural planktonic ecosystems as well as with unialgal cultures of diatoms. In the latter, the release of DOM was found to depend on the development stage of the phytoplankton population, day–night cycle, and growth-cycle phases. Examples are presented showing that, with this enclosure system, plankton succession can be observed up to 4 weeks at natural concentrations of ecosystem components; planktonic ecosystems can be collected from a coastal current in 40-m deep enclosures while maintaining the stratification in existence at the moment of enclosure; drift experiments can be performed in the open sea with planktonic ecosystems free of coastal influences (e.g., low nutrient concentrations), thereby allowing direct comparison between free and enclosed systems; and experiments with monocultures of diatoms can be run under nearly natural conditions, giving results that are similar to data obtained from natural systems.

The major part of the labile dissolved organic matter (DOM) included in the fluxes of material in marine planktonic ecosystems is released by phytoplankton cells. Studying release processes in the natural environment, i.e., "open sea," is as complicated as studying other ecological processes due to unevenly distributed ecosystem components moved by continuously changing advection. Results from laboratory experiments, on the other hand, can only be used to interpret natural conditions within limitations. Nonphysiological concentrations or artificial conditions that influence release processes often occur in the laboratory. Therefore, ecosystem research involving enclosure experiments represents a valuable bridge between extended investigations of the hydrodynamically ruled open water and laboratory experiments carried out under artificial conditions.

Since 1972, enclosure experiments have been performed to investigate several aspects of marine planktonic ecosystems, especially processes involving the release of dissolved organic substances by phytoplankton. Enclosures are used that are clean and impermeable, allowing investigation of phytoplankton populations under near-natural conditions over a period of 2–4 weeks.

Depending on the objectives and environments, enclosures of different lengths were used. For instance, optimum size depends on the number of trophic levels to be investigated (Kuiper et al. 1983).

In 1972, investigations of natural plankton communities were started. Because interactions within a natural complex ecosystem with successions of phyto- and zooplankton were observed to be too complicated to obtain quantitative relationships between concentration changes of dissolved organic substances and organisms (Brockmann et al. 1977a, 1979), the system was simplified and the next enclosure series involved phytoplankton monocultures. These experiments also investigated the degree of reproducibility of the system (Brockmann et al. 1977b).

Another important consideration with respect to working with enclosures is the comparability with nature. In addition to concentration ranges of different parameters at coastal sites, development within enclosures cannot be compared directly with development outside enclosures, even in areas with low tidal currents, as was documented in a series of experiments conducted in a southern Norwegian fjord (Brockmann et al. 1981, 1983a). A direct comparison is possible when drifting enclosures filled with a body of water remain for several days in the surrounding environment (Brockmann et al. 1984). The enclosure experiments reviewed in this paper are listed in Table 1.

Basic bag construction

The typical enclosure used in these studies consists of a seamless plastic tube 1 m in diameter (Brockmann et al. 1974, 1983a). Tubes of 5–40 m length were closed at the bottom, and several kilograms of weights were attached to keep the tube vertical. The tubes are open at the surface, attached to an aluminum ring supported by buoys (Fig. 1) or hung in a platform construction. The plastic foil consists of two combined layers, 100 µm of polyethylene on the inside and 30 µm of polyamide on the outside. This foil material has the advantage of being: one, impermeable to gases and dissolved metals; two, physiologically inert; three, flexible and heat permeable; four, light permeable (i.e., the colourless type) to about 90%; and, five, relatively cheap. Therefore, new foils can be used for each experiment, giving reproducible initial conditions. When working in coastal areas, the open enclosure is covered with an acrylglass plate to avoid pollution, e.g., from seabirds (Fig. 2).

Samples were collected from natural ecosystems using bottles or specially designed samplers (Brockmann and Hentzschel 1983) and from monoculture systems by tubes using vacuum (Brockmann et al. 1977b).

Analytical methods

Analytical methods are described briefly here. For greater detail, the reader should refer to the literature cited.

Table 1. Enclosure experiments.

Type	Location	Season	Depth of enclosure (m)	Duration (d)	Results	
					Dissolved organic matter (DOM)	Enclosure technique
Natural system	Heligoland harbour	Summer	4	28	• Indications of DOM release by phytoplankton	• Stable system • Plankton succession
Diatom monoculture	Heligoland harbour	Summer	4–5	11	• Quantification of DOM release by phytoplankton • Physiological control of DOM release	• Reproducible system • Frequent sampling for trace analysis
Natural system	Norway fjord	Early spring	40	12–23	—	• Constancy in changing environment
Natural system	North Sea (open sea)	Spring	15–25	10	• Utilization of DOM by phytoplankton	• Comparability with open systems

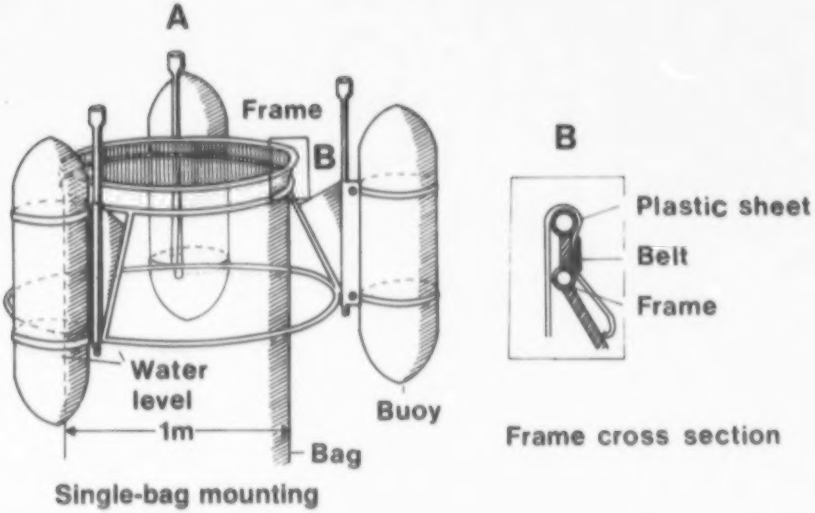

Fig. 1. Schematic of a single-bag mounting. The seamless plastic tube is fastened to an aluminum ring, which is supported by three buoys (A). The bags are fixed to the frame by a canvas belt, in a groove (B) (after Brockmann et al. 1983a).

- Samples were filtered mainly through glass-fibre filters (GF/C–Whatman, 1.2-µm retention ability) using a slight, constant vacuum (50 kPa).
- Filtrates were fixed with $HgCl_2$ (3.5% wt/vol), stored at 4°C (Brockmann et al. 1983b). Filters were frozen.
- Salinity was measured using probes calibrated by salinometer measurements.
- Nitrate was analyzed using an Technicon AutoAnalyzer following methods recommended by Technicon (Eberlein et al. 1983).
- For the analysis of dissolved carbohydrates, samples were filtered through 0.6-µm polyamide filters (Sartorius SM 19905) and fixed with $HgCl_2$ (see above).
- Total dissolved carbohydrates were analyzed using an AutoAnalyzer L-tryptophan–sulfuric acid method (Eberlein and Hammer 1980). The measurements, as glucose equivalents, also include oligo- and polysaccharides.
- Individual carbohydrates were analyzed after desalting by electrodialysis (ion-exchange membranes — Nepton membranes, Serva). Subsamples were hydrolyzed using 1.8 *N* HCl (100°C, 3.5 h) under N_2. Analysis was performed using an automatic sugar analyzer (Biotronik) (Mopper et al. 1980) or, for the first experiment, by gas chromatography of trimethylsilylesters (Brockmann et al. 1979).
- Total free amino acids were analyzed by cation exchange chromatography using a modified amino acid analyzer (Biotronik), fluorescence detection with *o*-phthaldialdehyde (Garrasi et al. 1979; Hammer and Luck 1987).

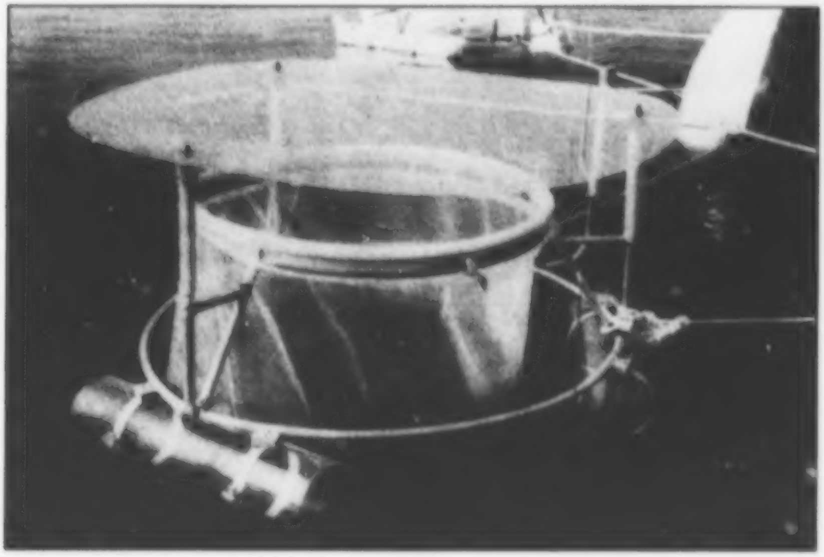

Fig. 2. Enclosure covered with an acrylglass plate.

- Total particulate carbohydrates were analyzed following the anthrone method (Brockmann et al. 1979).
- Plankton biomass was calculated from cell counts or individual counts, using conversion factors.
- Bacteria were estimated using the plate method, as colony-forming units, using marine agar (Brockmann et al. 1977b).
- Chlorophyll was estimated by spectrophotometric methods (Lorenzen 1967; Strickland and Parsons 1972) or using a Turner fluorometer calibrated by the spectrophotometric method.

Results and discussion

Complex natural ecosystem

In 1972, experiments were started using 4-m deep enclosures (3 m^3) in the sheltered Heligoland harbour in the German Bight (North Sea) to investigate succession within natural plankton populations during summer and accompanying compositional changes of dissolved organic substances.

In one of the first enclosures, which operated for 28 days, carbohydrates and free amino acids, as examples of dissolved organic substances, were analyzed (Brockmann et al. 1979). A succession of more than 20 different phytoplankton species was observed, of which five reached cell numbers over 100 000 dm^{-3} (Brockmann et al. 1977a). The calculated biomass of total phytoplankton and zooplankton was in the same range (Fig 3).

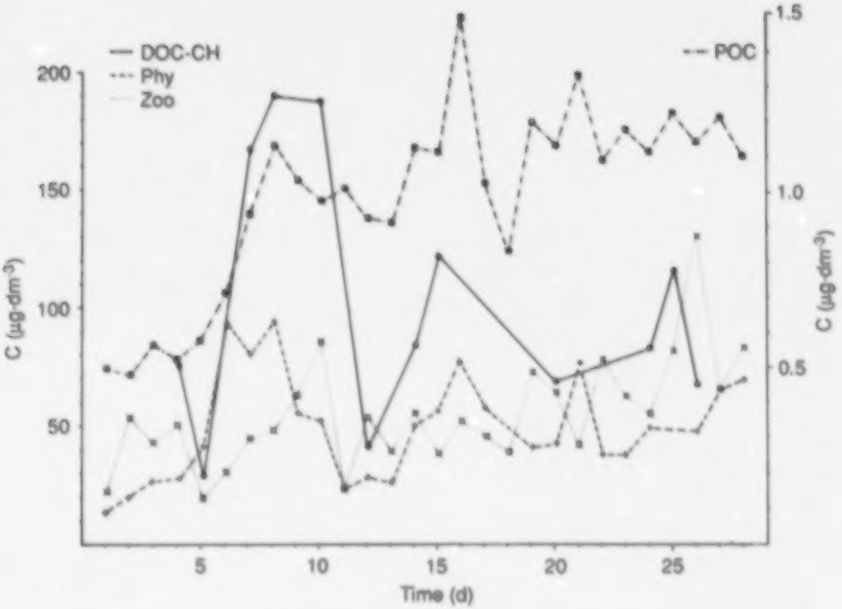

Fig. 3. Results from an enclosure experiment with a complex natural ecosystem. Note the difference in scale for particulate carbon (POC). Dissolved carbohydrates (DOC-CH) reached higher concentrations than the calculated biomass of phytoplankton (Phy) and zooplankton (Zoo) (after Brockmann et al. 1977a).

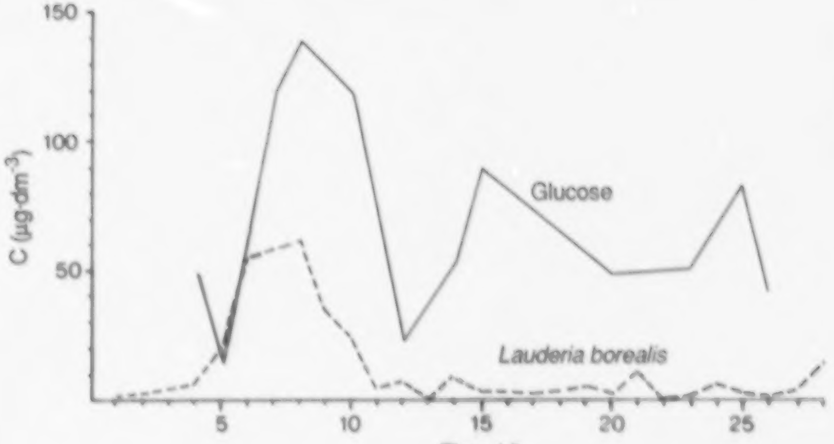

Fig. 4. Two components of an enclosed natural plankton ecosystem: *Lauderia borealis* carbon content calculated from cell numbers and dissolved total glucose.

One of the dominating phytoplankton species was a diatom, *Lauderia borealis*, which reached a biomass of 70 $\mu g\ C \cdot dm^{-3}$ (Fig. 4). The growth of *L. borealis* was limited by the depletion of silicate and ammonium. At the transition from the exponential to the stationary phase, the concentrations of carbohydrates, mainly glucose, increased to 140 $\mu g \cdot dm^{-3}$. At the same time, the measured particulate carbon content reached nearly 1 $mg \cdot dm^{-3}$. The grazing rates, calculated from the zooplankton stock, were in the same range as the calculated phytoplankton growth rates. Therefore, release of glucose by the scenescing phytoplankton population, as well as in the course of grazing, is probable. Glucose, which was released at a rate of 0.7 $\mu mol \cdot dm^{-3} \cdot d^{-1}$ decreased at the same rate, probably due to organotrophic uptake. The water temperature was about 15°C; therefore, biological processes were relatively fast. Other glucose peaks appeared and more than five other carbohydrates were detected during the development of the enclosed ecosystem.

During the experiment, 12 free dissolved amino acids were also detected, but because of rapid phytoplankton succession and zooplankton interactions with copepods, ciliates, and benthic larvae, it was not possible to give definite explanations for the release of the dissolved organic substances in this complex ecosystem with short-cycled fluxes of material.

Phytoplankton monocultures

To investigate the connections between phytoplankton development and the release of dissolved organic substances in more detail, enclosure experiments were performed with monocultures of one diatom species. Xenic phytoplankton cultures of *Thalassiosira* were used (Brockmann et al. 1977b). These experiments were also performed in the outer harbour of Heligoland. The water was taken 10 nautical miles (18.5 km) west of the island, pumped into plastic tubes that were sheltered in heavy textile bags, and transported below the surface to the Heligoland harbour (Fig. 5). Here, the water was filtered to remove algae, mixed, and put into three enclosures that were enriched with nutrients. The algal monocultures were subsequently inoculated.

The water in the bags was mixed artificially (within 10 $min \cdot cycle^{-1}$) so that only one representative sample had to be taken at a time from each enclosure. This allowed taking samples more frequently to investigate diurnal processes.

The water entered a central acrylglass tube, was driven downward by a propeller (<500 $circles \cdot min^{-1}$) within the tube, and welled up in the bag slowly until it entered the tube again (Fig. 6) (Brockmann et al. 1974). To check the reproducibility of the enclosure equipment, parallel experiments were performed with three enclosures, which were handled in the same way.

The enrichment of nutrients allowed investigating the exponential growth of *Thalassiosira rotula* for several days. During this time, nutrients were depleted (Fig. 7). There was good agreement between the exponential development of the two systems. This also includes the organotrophic bacteria, the growth of which was inhibited during the exponential phytoplankton development and increased when the phytoplankton numbers plateaued.

At the beginning of the stationary growth phase, the particulate carbon content increased in spite of unchanging cell numbers and nitrogen content per cell (Fig. 8) (Brockmann et al. 1983b). The maximum particulate carbon concentration was

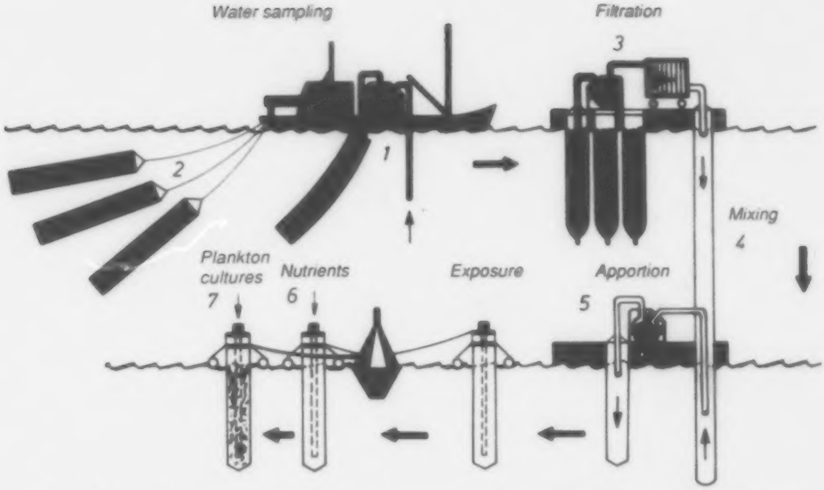
Water sampling
Filtration
3
2
1
Mixing
4
Plankton cultures
7
Nutrients
6
Exposure
Apportion
5

Fig. 5. Schematic of enclosure handling for monoculture experiments. A water sample of 4 m^3 was pumped (Flex-i-liner pump, Vanton, Fribourg, Switzerland) into plastic foil enclosures (1), which were protected by a Trevira cover and slowly towed (2) to the experimental site. Here, the water from the different enclosures was filtered (3) using plate filters (effective surface of 4 m^2, type 2/1250 Seitz, Bad Kreuznach, Germany) and mixed (4) in one large plastic tube to avoid deviations between parallel filled bags (5), which were exposed in frames (Figs 1 and 2). Often, a stirrer system was introduced to enable frequent and representative sampling with limited sample numbers. Finally, nutrients were added (6) and precultured phytoplankton inoculated (7).

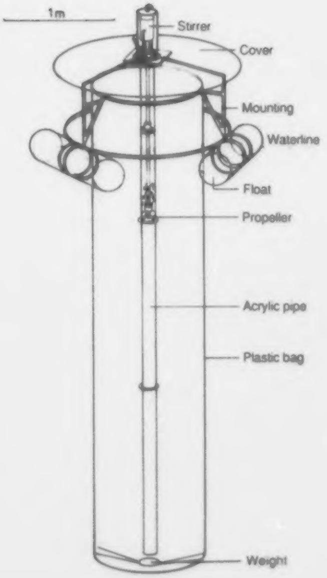
1m
Stirrer
Cover
Mounting
Waterline
Float
Propeller
Acrylic pipe
Plastic bag
Weight

Fig. 6. Enclosure with a stirrer for monoculture experiments (after Brockmann et al. 1974).

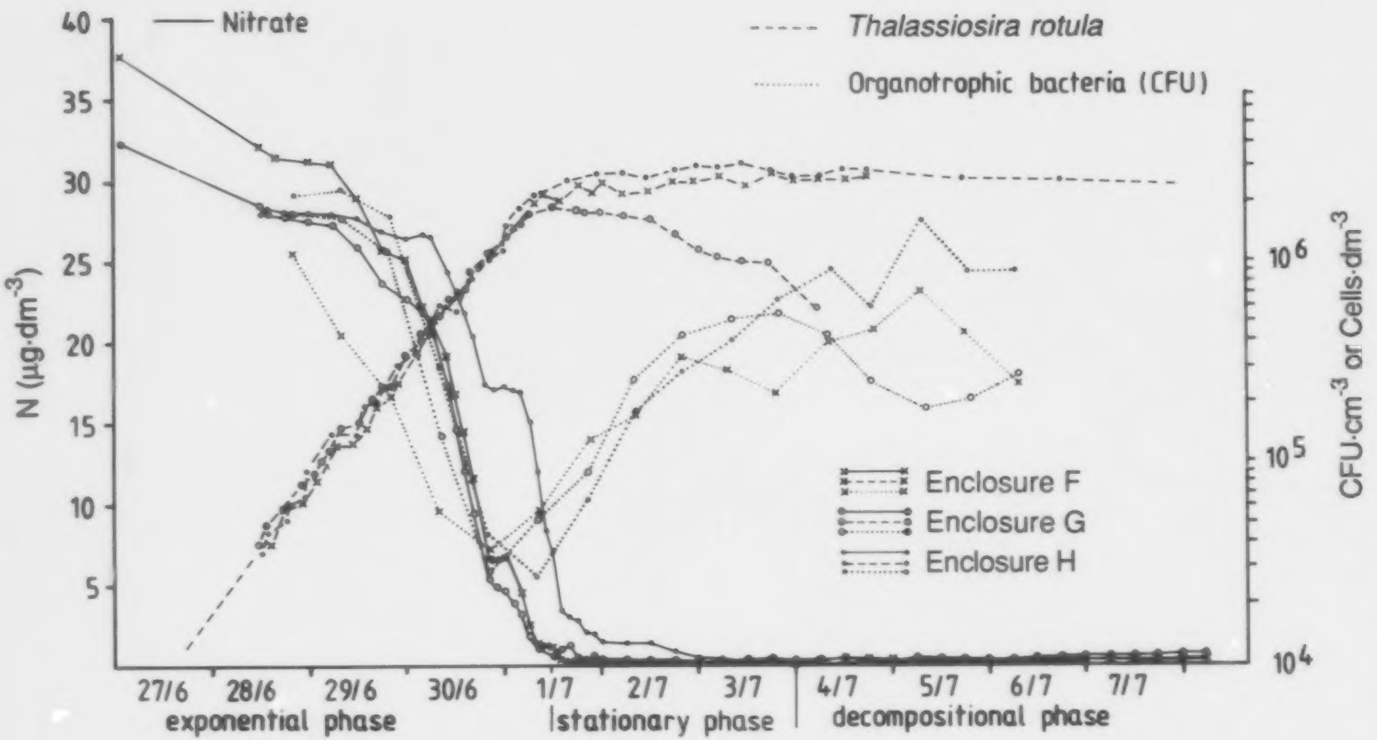

Fig. 7. Concentrations of nutrients, diatoms, and organotrophic bacteria (logarithmic scale) from three parallel experiments with xenic cultures of *Thalassiosira rotula*. Exponential, stationary, and decompositional phases are indicated.

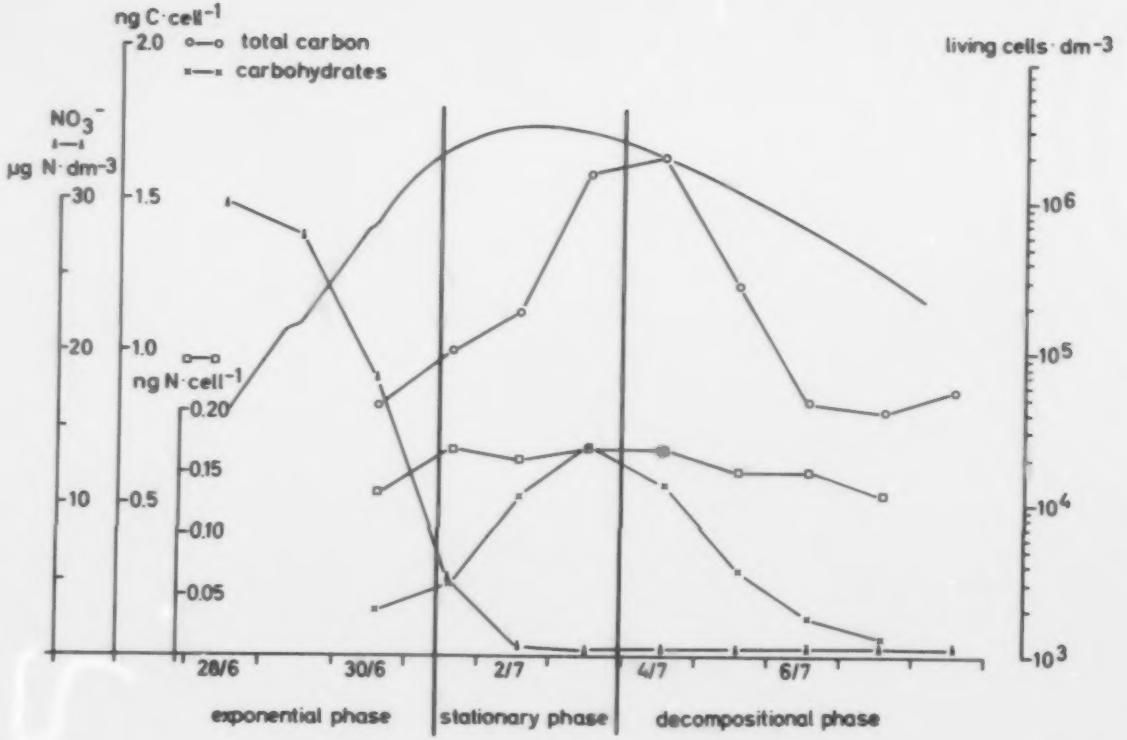

Fig. 8. Particulate components of a monoculture experiment with *Thalassiosira rotula*. The increase in particulate carbon during the stationary growth phase is shown (after Brockmann et al. 1977b).

observed 3 days after the end of the exponential growth phase. At the same time, particulate carbohydrates increases in similar quantities. In terms of number, the carbon content per cell increased from 0.8 to 1.7 ng per cell, at 0.15 ng N per cell. Of the total increase in carbon of 0.9 ng per cell, an increase of 0.7 ng carbohydrate C occurred during the stationary phase while biosynthesis continued. Because of a lack of nitrogen, proteins could not be synthesized and biosynthesis stopped at the level of the carbohydrates. Not all of the synthesized carbohydrates were stored within the cells, however, some were released.

One-third of total dissolved carbohydrates (substances with aldehyde or keto groups) could be identified after acid hydrolysis as individual monosaccharides. The dominating monosaccharide in the neutral carbohydrates (more than 90% of polymer compounds) was glucose, with more than 60% (molar). During the exponential phase, a higher percentage of the carbohydrates, mannose, and galactose was detected, which decreased during the stationary phase, but reappeared at higher concentrations during the decomposition phase. One reason for this change in composition of individual carbohydrates was that, during the stationary phytoplankton growth phase, storage polysaccharides, mostly glucose polymers, were released according to the slowdown of biosynthesis at the carbohydrate level. During the decomposition phase, structural polysaccharides were also released.

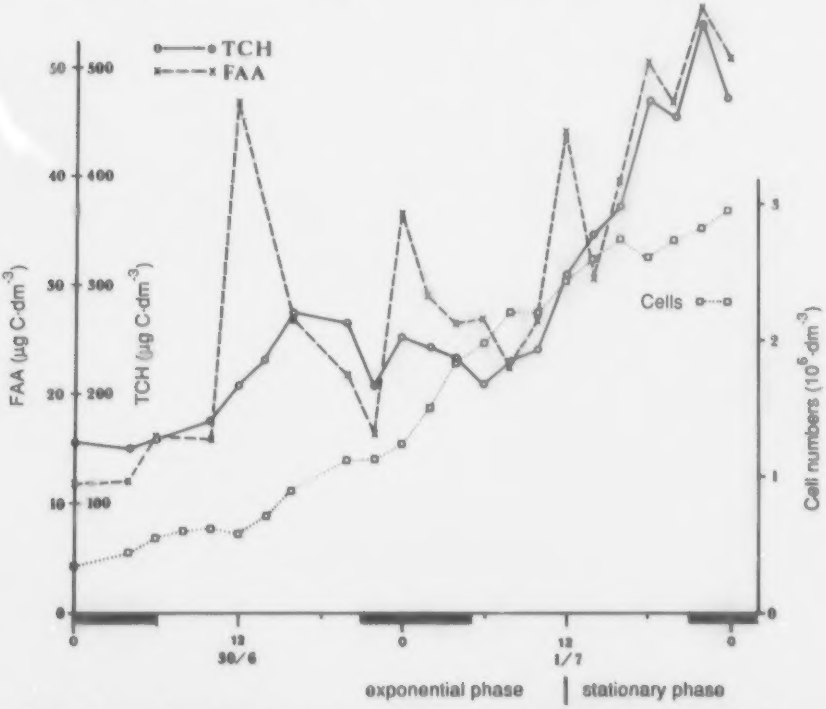

Fig. 9. Cell numbers, total dissolved carbohydrates (TCH), and total dissolved free amino acids (FAA) in an enclosure (enclosure H) with monocultures of *Thalassiosira rotula* (after Brockmann et al. 1977b; Hammer and Brockmann 1983; Eberlein and Brockmann 1986).

Concentrations of total dissolved carbohydrates and free amino acids increased during the exponential growth phase (Fig. 9) (Hammer et al. 1981; Eberlein and Brockmann 1986).

Sampling at a frequency of up to 2 h revealed diurnal fluctuations of cell numbers as well as concentrations of total carbohydrates and free amino acids. This means that the phytoplankton division was partly synchronous. Fluctuation of the concentration of dissolved organic substances was dependent on diatom metabolism and decomposition by organotrophic bacteria.

Based upon measurements taken very 6 h, it is evident that total carbohydrates were released mostly during the assimilation phase (Fig. 10). This process also continued during the stationary growth phase (Eberlein and Brockmann 1986). Furthermore, the measurements showed the reproducibility of enclosure experiments. The released carbohydrates were decomposed by organotrophic microorganisms at about the same rate. Therefore, the increases in concentrations detected represented only the net release rates.

Diurnal concentration changes of carbohydrates were also observed in monocultures of *Skeletonema costatum* (Eberlein et al. 1983). From these experiments, performed during different seasons and at different locations, the general significance of the findings was confirmed because similar daily concentration cycles of dissolved carbohydrates were found.

During the stationary phase of *T. rotula*, about 600 $\mu g \cdot dm^{-3} \cdot d^{-1}$ carbohydrate C

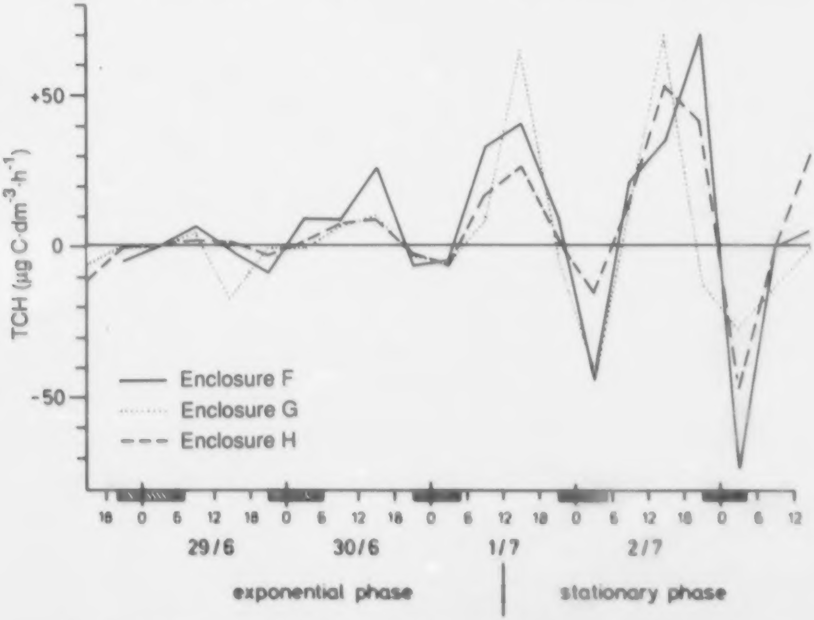

Fig. 10. Diurnal concentration changes of total carbohydrates of three parallel enclosures with monocultures of *Thalassiosira rotula*. Increases and decreases were calculated from concentrations estimated every 6 h (after Eberlein and Brockmann 1986).

was released, which comprised more than 50% of the particulate primary production at this time. During the exponential phase, this comprised between 14 and 28% of the particulate primary production. This means that the products of the incomplete biosynthesis were not only stored within the cells but were also released to the environment with about 30% of particulate primary production. At the beginning of the stationary phytoplankton growth phase, the increasing bacterial population caused faster uptake of carbohydrates, reaching the same rates of uptake as net release (Brockmann et al. 1977b).

The generation time of *Thalassiosira rotula* was 11.7 h. Because the division was mainly phased, short-time fluctuations in addition to diurnal activity changes could be measured by frequent sampling. Deviation of cell numbers from the logarithmic linear trend shows that most cells divided during morning, afternoon (1500 h), and evening (2000 h) (Fig. 11). Minimum division was observed at noon.

The release of dissolved organic substances also depended on the dividing

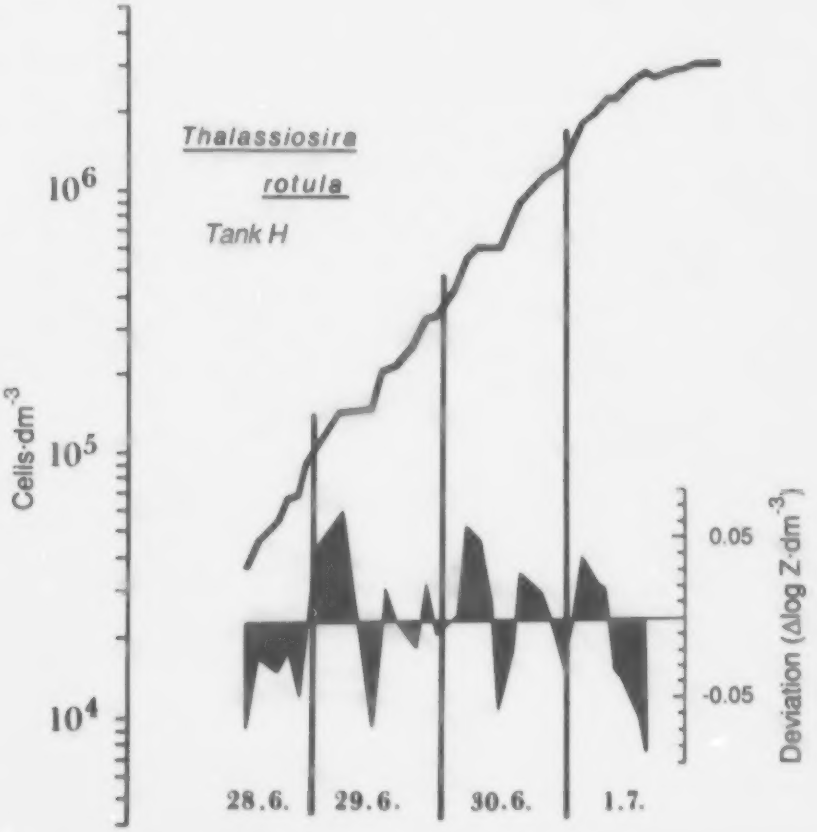

Fig. 11. Fluctuations in cell division of *Thalassiosira rotula*: cell numbers (upper curve) and deviations from the logarithm function (below).

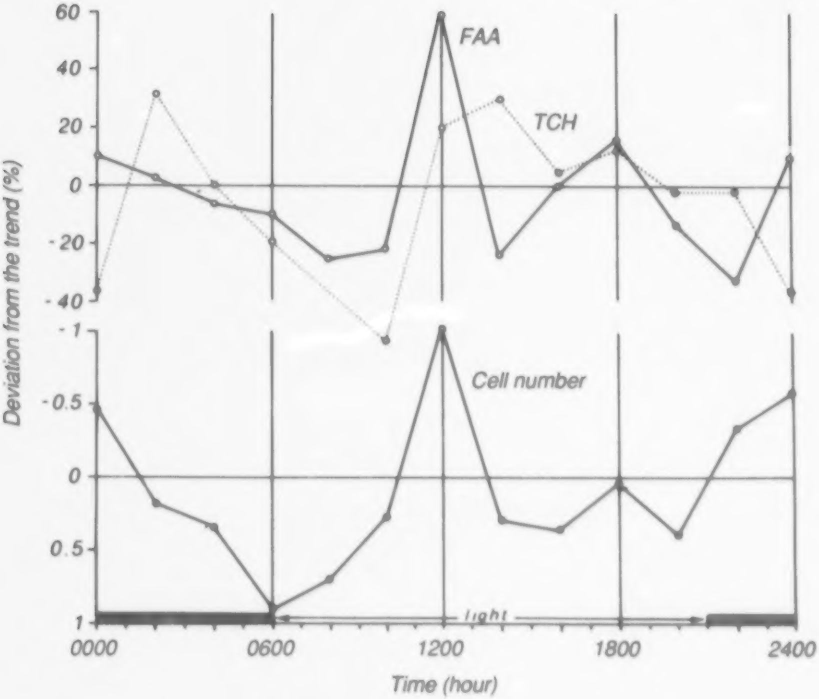

Fig. 12. Short-term fluctuations in dissolved organic substances (total carbohydrates (TCH) and free amino acids (FAA), shown as the deviation from the running means) and fluctuations in cell numbers of *Thalassiosira rotula* (deviations from the logarithm of cell numbers) (after Brockmann et al. 1977b: Hammer and Brockmann 1983; Eberlein and Brockmann 1986).

stages of the phytoplankton cells. Forming the deviations of the actual concentrations from fivefold overlapping means, especially reciprocal correspondence was found for the cell numbers and free amino acid concentrations (Hammer and Brockmann 1983) (Fig. 12). This means that during cell division in the morning, afternoon, and evening, fewer amino acids were released than during assimilation phases. Similar trends could be detected for the neutral carbohydrates (Eberlein and Brockmann 1986).

During the exponential growth phase of the diatom, short-chained free amino acids dominated the released amino acids. Thus, the C:N ratios of free amino acids decreased from 3.7:1 at the start to 3.1:1 (Hammer and Eberlein 1981, Hammer et al. 1981). During the stationary phase, more long-chained amino acids, such as glutamic acid, were released.

The question is whether these shifts in the release of dissolved organic substances during different phytoplankton growth phases found in enclosure experiments can also be confirmed by investigations in the open sea. Observed changes in the C:N ratio of free amino acids during the course of growth phases of a phyto-

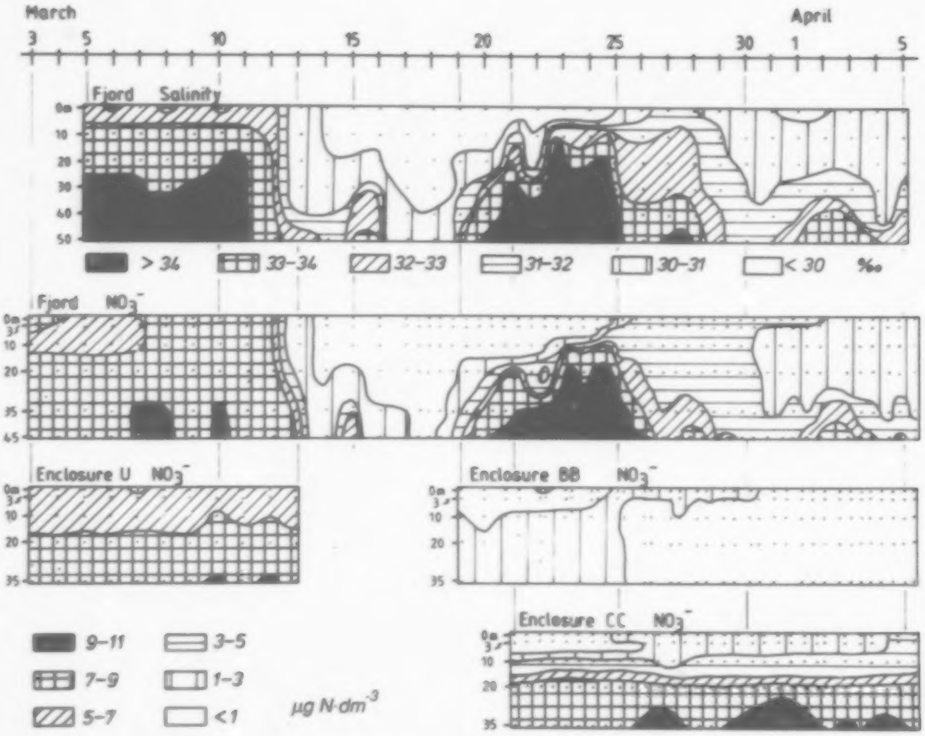

Fig. 13. Salinity and nitrate concentrations in an open Norwegian fjord (spring 1979, POSER I experiment) and nitrate concentrations in three enclosures started under different situations (after Brockmann et al. 1981, 1982; Kaffner et al. 1983).

plankton population were comparable with results obtained during the Fladen Ground Experiment in the open sea (Hammer et al. 1983; Hammer and Kattner 1986). During the exponential phase of the phytoplankton spring bloom, the C:N ratio of free dissolved amino acids was 3.16:1, shifting to 3.7:1 during the decomposition phase.

Comparability with nature

Normally, at coastal sites, it is difficult to compare ecosystem development within stationary enclosures with that occurring outside the enclosure because of advection in free-water ecosystems, e.g., by tidal currents or altering coastal currents. For example, during enclosure experiments conducted in 1979 in an open Norwegian fjord (Brockmann et al. 1981), a more or less continuous exchange of water masses, with some sudden hydrodynamic events (Fig. 13), was observed in the free water in spite of amphidromic conditions in this area, with tidal heights below 0.3 m. During these events, the surface water can be exchanged within 12 h down to a depth of more than 80 m, as revealed by salinity measurements. The different water bodies also exhibited different nutrient concentrations and plankton. Nitrate concentrations in the open fjord, for instance, changed with water mass exchange, but within the enclosures, nitrate was influenced only by the activity of the enclosed ecosystems. The vertical stratification captured at the time of bag filling was preserved until the end of the experiment (Brockmann et al. 1982; Kaffner et al. 1983) in spite of permanent temperature adaptations to the changing environment.

A direct comparison between ecosystem development in enclosures and in free water can be made using drifting enclosures. To perform drift experiments with enclosures, a ringlike float was developed with a diameter of 6 m (Fig. 14). It was constructed from fibreglass plastic with a 2-m high aluminum frame designed to carry three bags. In the North Sea, 25-m long enclosures were used. The bags were prepared from flat, rolled-up tubes, closed at the bottom, and attached to weights (about 10–20 kg). The collapsed tubes were lowered, together with aluminum rings to which the upper open ends of the tubes were fixed (Figs 1 and 2), to a depth of 25 m. Then the bags were filled by raising them using a winch. Three bags could be filled within 1 h, so they could be taken as replicates.

To study phytoplankton development at low nutrient concentrations after the spring bloom, drift experiments were performed in May 1982 with enclosures in the eastern North Sea. This procedure was also employed to avoid coastal influences, such as discharges or upwelling. As a result of a calm weather period, the enclosures were allowed to drift for 10 days (Fig. 15).

Water columns with extremely low nutrient concentrations were included in the mixed layer (Brockmann et al. 1984). Nitrate was below 1 $\mu g \cdot dm^{-3}$, ammonium below 0.4 $\mu g \cdot dm^{-3}$, phosphate below 0.05 $\mu g \cdot dm^{-3}$, and silicate below 0.5 $\mu g \cdot dm^{-3}$. One of the dominant species was *Chrysochromulina mactra*, which at the beginning of the experiment was growing near the surface at a depth of about 12 m. This population was trapped by a second thermocline, which was formed during the calm weather by irradiation. This process could also be observed in the open sea, as discerned from chlorophyll measurements (Fig. 16). After 5 days, the drift station departed from the water body, as indicated by a change in salinity. During this

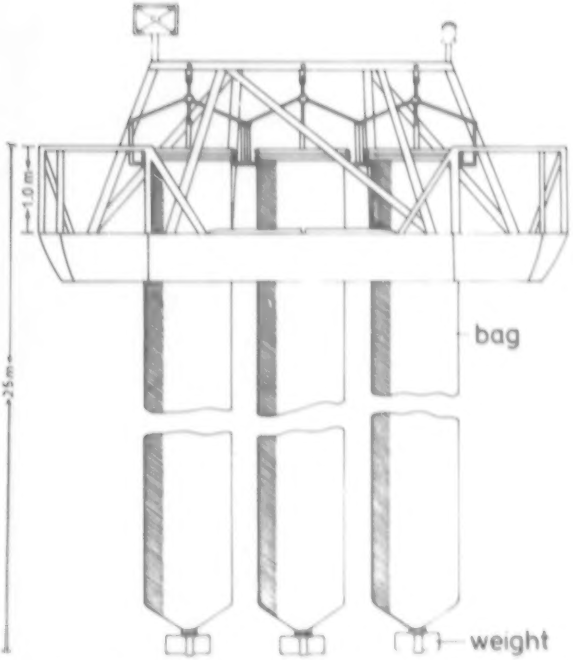

Fig. 14. Schematic of drift station for three enclosures. The float is manufactured from fibreglass. The enclosures are fixed to the 2-m high aluminum frame. The station is equipped with a radar reflector and strobe light.

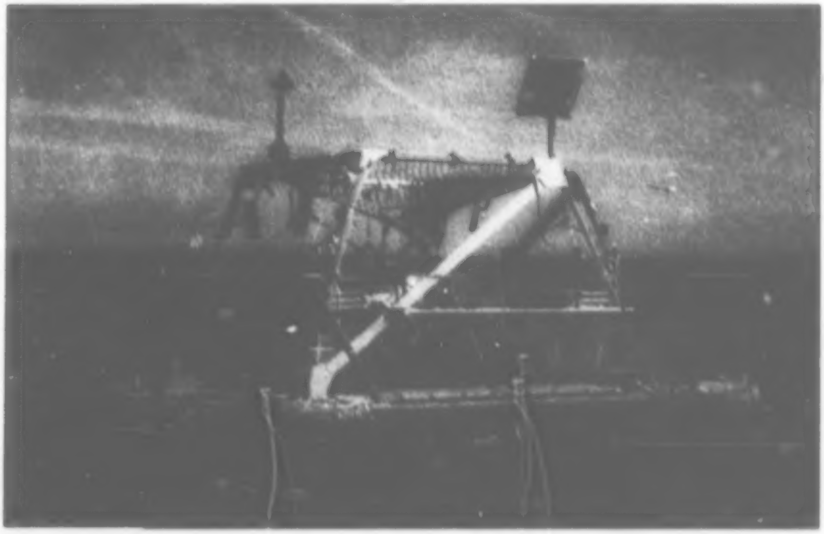

Fig. 15. Drifting enclosure station in the North Sea.

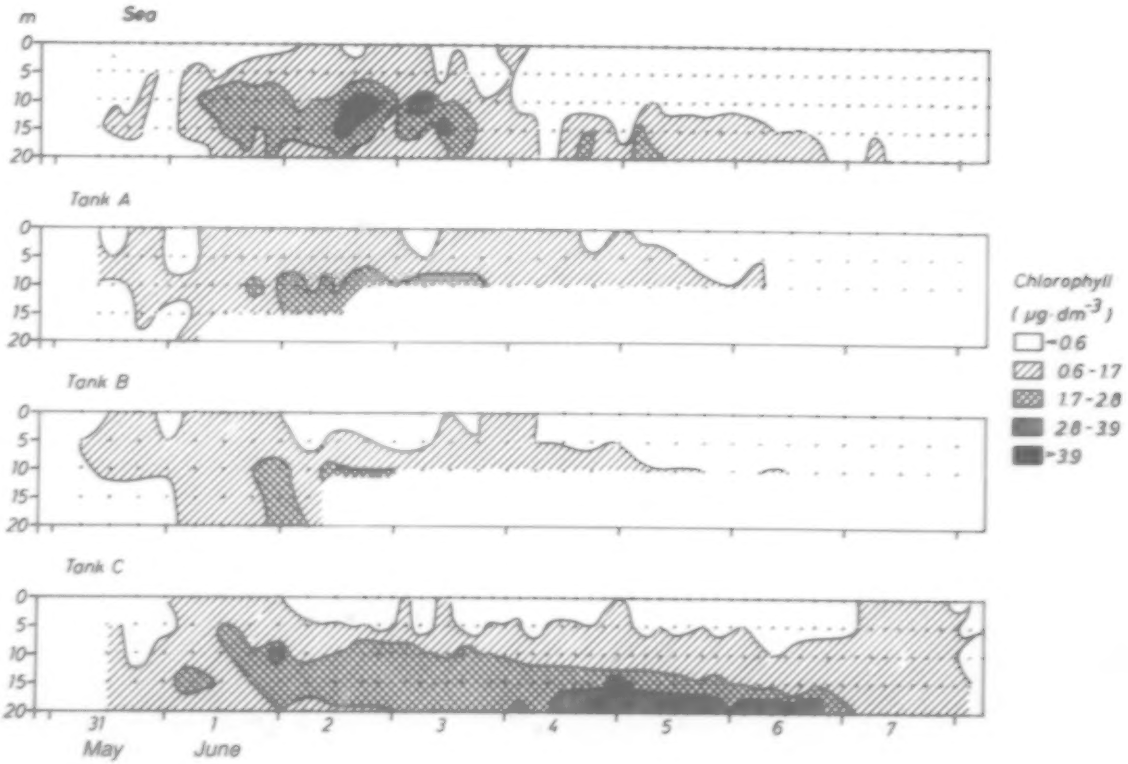

Fig. 16. Chlorophyll concentrations in the sea and three enclosures during a drift experiment (after Brockmann et al. 1984).

experiment, nitrogen and phosphorus requirements were met by available dissolved organic compounds.

Frequent measurements of chlorophyll, nutrients, and free amino acid concentrations, both in the enclosures and in the open sea, confirmed the parallel development in both.

Conclusions

The results show that using the enclosure system described here allows one to obtain results that are reproducible under given environmental conditions and comparable with natural free systems for shorter periods and lower trophic levels.

Acknowledgments

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Brockmann, U.H., Hentzschel, G. 1983b. Samplers for enclosed stratified water columns. Marine Ecology, Progress Series, 14, 107–109.

Brockmann, U.H., Eberlein, K., Junge, H.D., Trageser, H., Trahms, K.J. 1974. Einfache Folientanks zur Planktonuntersuchung in situ. Marine Biology, 24, 163–166.

Brockmann, U.H., Eberlein, K., Hosumbek, P., Trageser, H., Maier-Reimer, E., Schöne, H.K., Junge, H.D. 1977a. The development of a natural plankton population in an outdoor tank with nutrient-poor sea water — I: Phytoplankton succession. Marine Biology, 43, 1–17.

Brockmann, U.H., Eberlein, K., Hetzschel, G., Schne, H.K. 1977b. Parallel plastic tank experiments with cultures of marine diatoms. Helgolaender Wissenschaftliche Meeresuntersuchungen, 30, 201–216.

Brockmann, U.H., Eberlein, K., Junge, H.D., Maier-Reimer, E., Siebers, D. 1979. The development of a natural plankton population in an outdoor tank with nutrient-poor sea water — II: Changes in dissolved carbohydrates and amino acids. Marine Biology, Progress Series, 1, 283–219.

Brockmann, U.H., Koltermann, K.P., Dahl, E., Dahle, A., Eberlein, K., Gaertner, A., Gassmann, G., Hammer, K.D., Jahnke, J., Kattner, G., Krause, M., Kuiper, J., Laake, M., Nagel, K. 1981. Water exchange in Rosfjorden during spring 79: a detailed account of physical, chemical and biological variations. *In* Saetre, R., Mork, M., ed., The Norwegian coastal current (vol. 1). University of Bergen, Bergen, Norway. 93–130.

Brockmann, U.H., Kattner, G., Dahl, E. 1982. Plankton spring development in a south Norwegian fjord. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 195–204.

Brockmann, U.H., Dahl, E., Kuiper, J., Kattner, G., 1983a. The concept of POSER (Plankton Observation with Simultaneous Enclosures in Rosfjorden). Marine Ecology, Progress Series, 14, 1–8.

Brockmann, U.H., Ittekkot, V., Kattner, G., Eberlein, K., Hammer, K.D. 1983b. Release of dissolved organic substances in the course of phytoplankton blooms. *In* Sündermann, J., Lenz, W., ed., North Sea dynamics. Springer-Verlag, Berlin, Germany. 530–548.

Brockmann, U.H., Jahnke, J., Hammer, K.D. 1984. A bloom of *Chrysochromulina* cf. *mactra* Manton in drifting enclosures in the North Sea. *In* Report of a Special Meeting on the Causes, Dynamics and Effects of Exceptional Marine Blooms and Related Events, 4–5 October. International Council for the Exploration of the Sea, Copenhagen, Denmark. B3, 21 pp.

Eberlein, K., Brockmann, U.H. 1986. Development of particulate and dissolved carbohydrates in parallel enclosure experiments with monocultures of *Thalassiosira rotula*. Marine Ecology, Progress Series, 32, 133–138.

Eberlein, K., Hammer, K.D., 1980. Automatic determination of total carbohydrates in sea water. Fresenius Zeitschrift fuer Anal. Chemie, 301, 17–19.

Eberlein, K., Hammer, K.D., Brockmann, U.H., Kattner, G. 1983. Dynamics of carbohydrate development in tank experiments with cultures of *Skeletonema costatum* and *Thalassiosira rotula*. *In* Sündermann, J., Lenz, W., ed., North Sea dynamics. Springer-Verlag, Berlin, Germany. 549–558.

Hammer, K.D., Brockmann, U.H. 1983. Rhythmic release of dissolved free amino acids from partly synchronized *Thalassiosira rotula* under nearly natural conditions. Marine Biology, 74, 305–312.

Hammer, K.D., Eberlein K. 1981. Parallel experiments with *Thalassiosira rotula* in outdoor plastic tanks: development of dissolved free amino acids during an algae bloom. Marine Chemistry, 10, 533-544.

Hammer, K.D., Kattner, G. 1986. Dissolved free amino acids in the marine environment: a carbon to nitrogen ratio shift during diatom blooms. Marine Ecology, Progress Series, 31, 35–45.

Hammer, K.D., Brockmann, U.H., Kattner, G. 1981. Release of dissolved free amino acids during a bloom of *Thalassiosira rotula*. Kieler Meeresforschungen Sonderheft, 5, 101–109.

Hammer, K.D., Eberlein, K., Kattner, G., Brockmann, U.H. 1983. Fluctuations of dissolved amino acids: a comparison of natural and enclosed phytoplankton populations in the North Sea. *In* Sündermann, J., Lenz, W. ed., North Sea dynamics, Springer-Verlag, Berlin, Germany. 569–572.

Kattner, G., Hammer, K.D., Eberlein, K., Brockmann, U.H., Jahnke, J., Krause, M. 1983. Nutrient and plankton development in Rosfjorden in enclosed ecosystems captured from changing water bodies during POSER. Marine Ecology, Progress Series, 14, 29–43.

Kuiper, J., Brockmann, U.H., Groenewoud, H. van het, Hoornsman, G., Hammer, K.D. 1983. Influences of bag dimensions on the development of enclosed plankton communities during POSER. Marine Ecology, Progress Series, 14, 9–17.

Lorenzen, C.J. 1967. Determinations of chlorophyll and pheopigments: spectrophotometric equations. Limnology and Oceanography, 12, 343–346.

Mopper, K., Dawson, R., Liebezeit, G., Ittekkot, V. 1980. Monosaccharide spectra of natural waters. Marine Chemistry, 10, 55–66.

Strickland, J.D.H., Parsons, T.R. 1972. A practical handbook of seawater analysis. Journal of the Fisheries Research Board of Canada, 167, 1–310.

Use of Enclosures for Assessing the Effects of Pesticides in Freshwater Aquatic Ecosystems

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A methodology for evaluating the effects of pesticides in an aquatic environment was developed using model compounds, such as permethrin, methoxychlor, atrazine, and diuron. This method made use of enclosures, or limnocorrals, as a means of isolating sections of a body of water into separate, replicable compartments that were treated with pesticides in a manner that would pose minimal hazard to the larger water system. The technique demonstrated replication of pesticide-induced effects, both within and between seasons; the ability to observe toxicity or the lack of it with respect to a large number of organisms at one time as well as the integrated impact on the whole system; and recovery from these effects. The technique demonstrated changes in diversity and interactions caused by selectivity of pesticide action as well as adaptations in the system that compensate, in part, for the effects of the pesticide. It was also possible to observe a dose–response relationship in both the degree of the effect and the response time. These observations allow one to establish acceptable-effect concentrations by interpolation and suggest approaches for formulating regulatory guidelines.

Enclosures or limnocorrals have become widely used as a mesocosm method or technique for studying the effects of a variety of substances in aquatic ecosystems. Although much of the early work in this area was devoted to studying ecological processes (Strickland and Terhune 1961; Whittaker 1961; Beyers 1962; Goldman; 1962), recent work has focused on the impact of chemical substances. These range from nutrients (Lack and Lund 1974; Beers et al. 1977; Blinn et al. 1977; Leah et al. 1978; Twinch and Breen 1981), metals (Davies and Gamble 1979; Marshall and Mellinger 1980), and oil products (Menzel and Case 1977) to pesticides (Solomon et al. 1980, 1985, 1986; Shires 1983; Kaushik et al. 1985; Herman et al. 1986; Stephenson et al. 1986). A large body of work on the impact of these types of chemicals in pond ecosystems also exists, but, with the exception of recent work on oil

(Franco et al. 1984; Giddings et al. 1984) and atrazine (Larsen et al. 1986), it is not discussed in this review.

The use of enclosures is based upon two philosophies. The first is the perceived need to assess the effects of a potentially toxic substance in the field, either under actual conditions of use or under circumstances during which it may come into contact with biota. This philosophy is based on the dogma (essentially an admission) that one cannot duplicate all environmental conditions in the laboratory. Laboratory assessments, therefore, can only act as a guide to developing hypotheses that must be subjected to testing in field assays. This dogma is widely accepted and is the *raison d'être* for the fieldwork of many environmental toxicologists as well as those in the applied agricultural sciences.

Historically, such field observations were normally made after contamination by or the release of a toxic substance. Although these studies have added much to understanding the processes whereby such substances are distributed in the environment and cause harm, they have done little to predict or prevent this damage from occurring in the first place. The need for systematic field trials of new crop varieties or agricultural chemicals has lead to similar assessments of environmental toxicants.

The results of these assessments have begun to be accepted by regulatory authorities as well as those who use or produce these substances. Another sound reason for using field assessments in natural settings is to account for the phenomenon of homeostasis or self-regulation. Most natural systems have a considerable degree of redundancy and are able to compensate in response to exogenous changes. Thus, most systems tend to return to a norm after being disrupted, even though this norm may be a progression or succession of events driven by seasonal or climatic forces.

Another philosophy inherent in the use of field assessment is opposite to the traditional and reductionist approach founded in the single-organism assay. This approach, as related by the late Professor Reigler of McGill University, involves a number of steps that start at a general or ecosystem level and terminate at the single-organism or biochemical process. The first step involves defining the system and describing its normal behaviour and properties. This is followed by searching for cause and effect after perturbation with the substance or process being studied. Consistent and repeatable effects may then be linked with a cause via an explanatory theory. This theory forms the basis for synthesizing hypotheses that may then be tested in an acceptable scientific manner.

Pesticides are a class of toxins that normally have some degree of selectivity with respect to their toxicity. Thus, they offer the ecotoxicologist an ideal tool for developing ecotoxicological methods. This usefulness is extended by the relatively large body of information available on the mode or mechanism of action of many pesticides as well as the susceptibility of a number of species to their toxic effects. Pesticides, as a class of chemicals, are useful as model toxins, both from the scientific point of view and from the point of view of establishing regulatory guidelines to control their use.

This review focuses on the results obtained by several groups working with a variety of pesticides and other toxic substances.

Materials and methods

Enclosures larger than 10 m^3 are usually constructed from a flexible plastic. Corrals used in this study were made from nylon-reinforced polyvinyl-chloride plastic supported at the surface by floats and weighted at the base (Solomon et al. 1980). The size of corrals used for pesticide studies has varied from the 1-m^3 stainless steel enclosures used by Shires (1983) to the 1 000 m^3 [illegible] enclosures used in this study. A design innovation of corrals used to study [illegible] insoluble compounds has been the use of a liner made from 152-µm thick polyethylene. This has permitted any adsorbed pesticide remaining at the end of the experiment to be removed (Solomon et al. 1986).

Limnocorrals have commonly been installed in water at depths similar to the diameter or width of the enclosure, e.g., in 4–4.5 m of water in the case of 125-m^3 corrals (Solomon et al. 1980). As they commonly cover a portion of the bottom sediments of the lake, it is necessary to ensure an adequate seal between the base of the limnocorral and the sediments — visual inspection by a Scuba diver is sufficient.

In practice, enclosures and other mesocosms have normally been treated with pesticides or other toxicants in replicates of at least two. This study involved three treatments at each concentration as well as three untreated corrals as reference enclosures. Pesticides were applied in two ways, by forced mixing into the water column (Solomon et al. 1980) and surface application (Larsen et al. 1986; Solomon et al. 1986).

Enclosures and mesocosms were sampled before and after pesticide application. Water chemistry, dissolved oxygen, secchi disk, and temperature were measured and samples were collected to enumerate the organisms in the systems. To compensate for the absence of the normal input of inorganic nutrients into the corrals, nitrogen and phosphorus, in the form of phosphoric acid and sodium nitrate, were added at weekly intervals after pesticide treatment. Monitoring of levels of sodium in the enclosure also reveals the occurrence of leaks, if any. Additional details regarding installing, treating, and sampling methods used in this study have been reviewed in Kaushik et al. (1986) and are not described further here.

Many pesticides are contaminants of aquatic ecosystems. Those of short persistence are more likely to enter through direct contamination, whereas those with longer persistence and higher water solubility may enter indirectly via soil erosion, runoff, and groundwater. Pesticides in this category have been reported in surface waters that drain into the Great Lakes (Baker 1983) and, as such, are of potential concern.

The pesticides used in this study were chosen for their known toxic effects. The insecticides methoxychlor and permethrin are known, from laboratory studies, to be toxic to zooplankton. These insecticides are of relatively short persistence, suggesting that their effects in the system would probably be short term and probably a direct result of acute toxicity. The herbicides atrazine and diuron are of relatively longer persistence and higher water solubility, but are known to be comparatively less toxic to zooplankton based on laboratory assays. Instead, they are inhibitors of photosynthesis in terrestrial plants and some algae (Torres and O'Flaherty 1976; Stratton 1984) and are, therefore, expected to affect primary production initially and, through indirect and relay effects, organisms in other trophic levels.

Results and discussion

Toxicity and recovery

Acute toxic effects of pesticides and other substances have been readily observed in mesocosms. Permethrin was toxic to all crustacea in the treated enclosures, even at concentrations as low as 0.5 $\mu g \cdot L^{-1}$ (Kaushik et al. 1985). The effects of toxicity were rapidly observed in population numbers that normally showed a precipitous decline within 24 h of treatment. Similarly, methoxychlor was toxic to crustacea at concentrations of 300, 50, 30, and 5 $\mu g \cdot L^{-1}$ (Stephenson et al. 1986). Methoxychlor had no toxic effect on rotifers, the numbers of which were consistently higher than, if not significantly different from, those in the control enclosures. Permethrin also had little effect on rotifers, except at high concentrations (Kaushik et al. 1985). Methoxychlor also had some effect on phytoplankton numbers (Fig. 1).

Although these effects were not as obvious, or as immediate, as those on the zooplankton, they were consistent between replicate enclosures and were statistically significant at times after treatment and at concentrations as low as 5 $\mu g \cdot L^{-1}$ for the Cryptophyta group. Although methoxychlor has been reported to have toxic effects

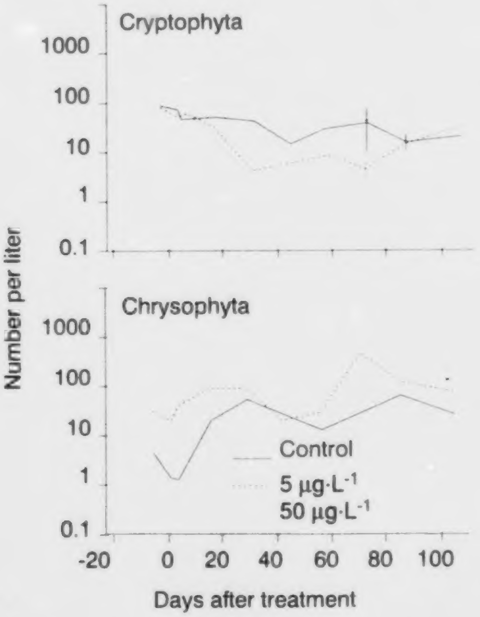

Fig. 1. The effect on phytoplankton of two concentrations of methoxychlor (5 and 50 $\mu g \cdot L^{-1}$) applied with mixing to 125-m^3 enclosures in 1981. The points represent means of three enclosures and the vertical lines indicate the standard deviation where the analysis of variance indicated significant differences between reference and treated enclosures ($P < 0.05$). Treatment was on day 0. (Redrawn from data of Stephenson et al. 1986.)

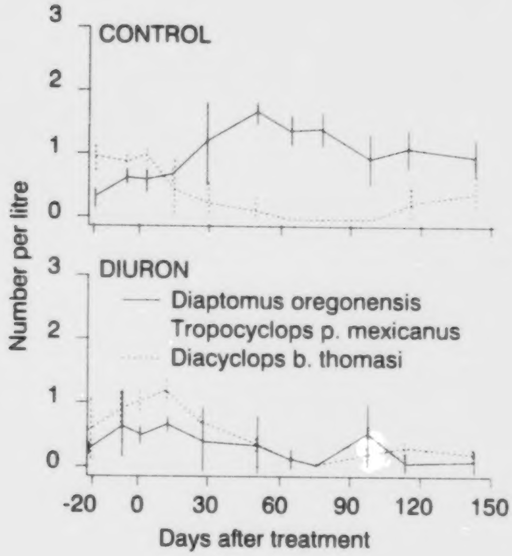

Fig. 2. The effect on three species of zooplankton of surface application of diuron at 500 µg·L^{-1} in 125-m^3 enclosures in 1982. The points represent means of three enclosures and the vertical lines indicate one standard deviation.

on algae in laboratory assay systems, these occur at concentrations one to two orders of magnitude higher than those in the enclosures (Butler 1963). This suggests the possibility that the effects may be indirect and may be caused by changes in the grazing patterns of the zooplankton.

In the case of the herbicides diuron and atrazine, no immediate acute toxicity was noted in the crustacea even though the concentrations used were several orders of magnitude higher than for the insecticides. Long-term decreases in the numbers of some zooplankton, such as *Diaptomus oregonensis* (Fig. 2), may be the result of chronic toxicity, but may also be the result of changes in food availability. Diuron caused a rapid reduction in the numbers of several species of large phytoplankton, such as *Tetraedron minimum* (Fig. 3), which may be important as a source of food for *D. oregonensis*. As was the case for diuron, atrazine also caused reductions in the numbers of some zooplankton (Fig. 4). *Diaptomus oregonensis* again appeared to be susceptible either to the chronic effects of the material or to changes in food availability. Atrazine also caused decreases in the numbers of phytoplankton (Fig. 5), although some species (*Chlamydomonas* spp and *Rhodomonas minuta*) seemed to be less sensitive and actually increased in numbers compared with the reference enclosures.

Recovery of the population to numbers similar to those in the reference enclosures appeared to be quite rapid (30–60 d) for those populations exposed to insecticides except at very high concentrations. The insecticides were not persistent (2–10 d half-lives) and their effects on biota were acute in nature. Changes induced

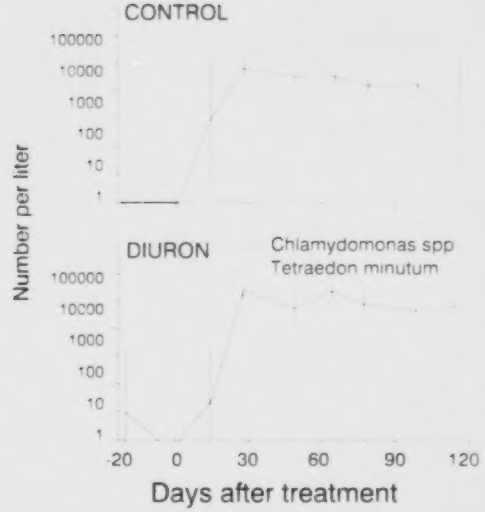

Fig. 3. The effect on two species of phytoplankton of surface application of diuron at 500 µg·L⁻¹ in 125-m³ enclosures in 1982. The points represent means of three enclosures and the vertical lines indicate one standard deviation.

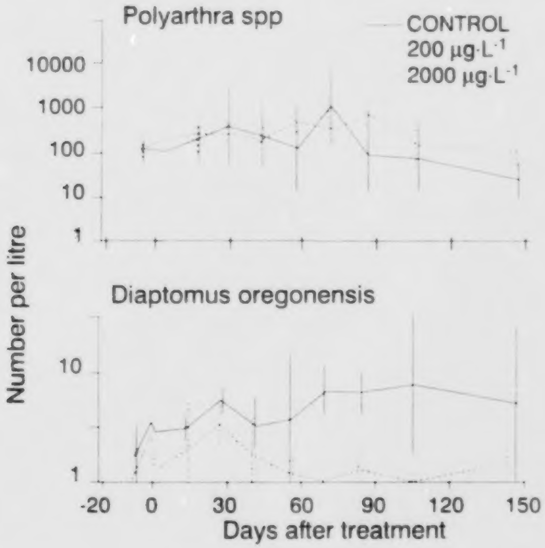

Fig. 4. The effect on two species of zooplankton of atrazine applied at 2 000 and 200 µg·L⁻¹ with mixing in 125-m³ enclosures in 1981. The points represent means of three enclosures and the vertical lines indicate one standard deviation.

by the herbicides were evident less rapidly and were long term in nature in keeping with the half-life of both compounds in this system (<1 year).

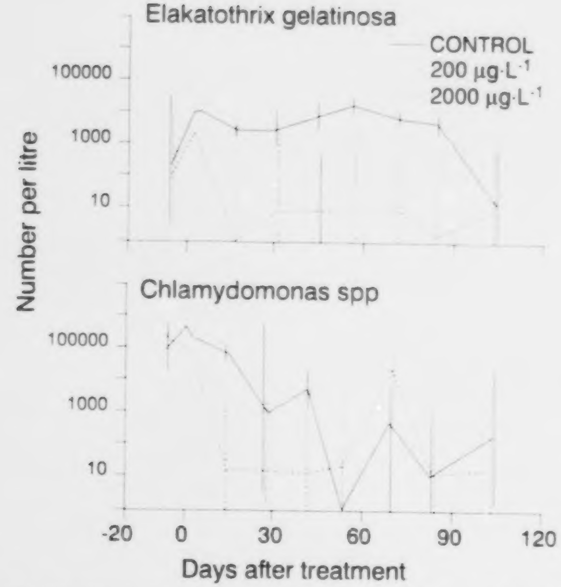

Fig. 5. The effect on two species of phytoplankton of atrazine applied at 2 000 and 200 $\mu g \cdot L^{-1}$ with mixing in 125-m^3 enclosures in 1981. The points represent means of three enclosures and the vertical lines indicate one standard deviation.

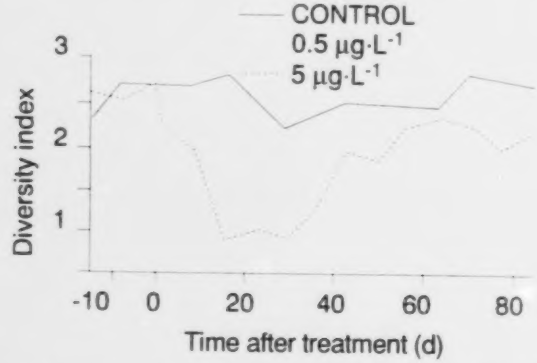

Fig. 6. The effect of permethrin applied with mixing at 0.5 $\mu g \cdot L^{-1}$ (low) and 5 $\mu g \cdot L^{-1}$ (high) on zooplankton diversity as measured by the Shannon Weaver index in 125-m^3 enclosures in 1980. (Redrawn from data of Kaushik et al. 1985.)

Diversity

The effects noted with respect to numbers of organisms are also reflected in measurements of diversity. Permethrin (Fig. 6) caused a short-term decrease in the diversity index, which returned to levels similar to those measured in the reference enclosures at about the same time as population numbers recovered. Methoxychlor

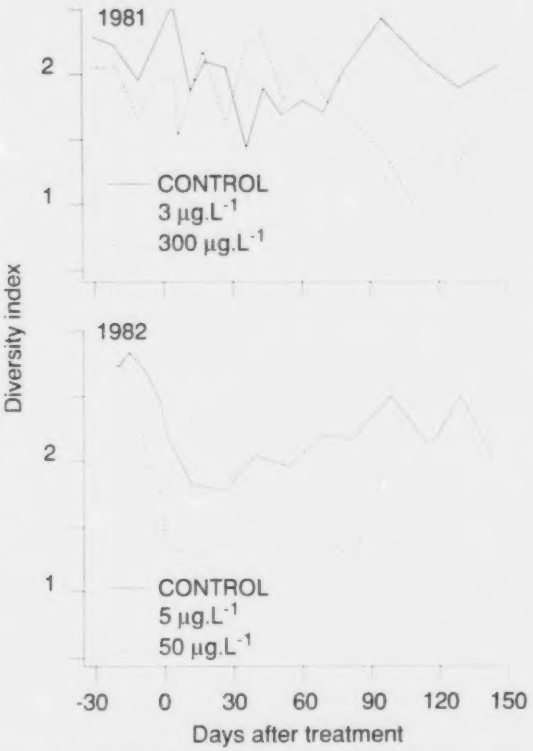

Fig. 7. The effect of methoxychlor applied as a surface application and on zooplankton diversity as measured by the Shannon Weaver index in 125-m^3 enclosures. (Redrawn from data of Stephenson et al. 1986.)

(Fig. 7) caused significant short-term changes in the diversity of zooplankton at 5 and 50 μg·L^{-1}, but not at 3 or 300 μg·L^{-1}. These studies were carried out in different years, but with the consistency of three replicates, the results are deemed comparable. Numbers of zooplankton did not change much at 3 μg·L^{-1}, but the decrease in numbers at 300 μg·L^{-1} was significant and long term in nature (Stephenson et al. 1986). The lack of sensitivity of the diversity index as a criterion of effect at 300 μg·L^{-1} is probably a result of the insensitivity inherent in all diversity indices at very low numbers of individuals.

The effect of diuron on the number of taxa of phytoplankton was marked and of longer duration (Fig. 8). The longer duration of the difference is clearly related to the long half-life of the material and is consistent with the observations on numbers of individual species. The fact that this change in the number of phytoplankton taxa present had no impact on the diversity of zooplankton (Fig. 8) suggests that, in general, these organisms are able to adapt to changes in both phytoplankton numbers and species present. This is not altogether surprising if one accounts for the differences in seasonal abundance of phytoplankton that occur and are tolerated by zooplankton. The decrease in the numbers of *D. oregonensis* in enclosures treated with

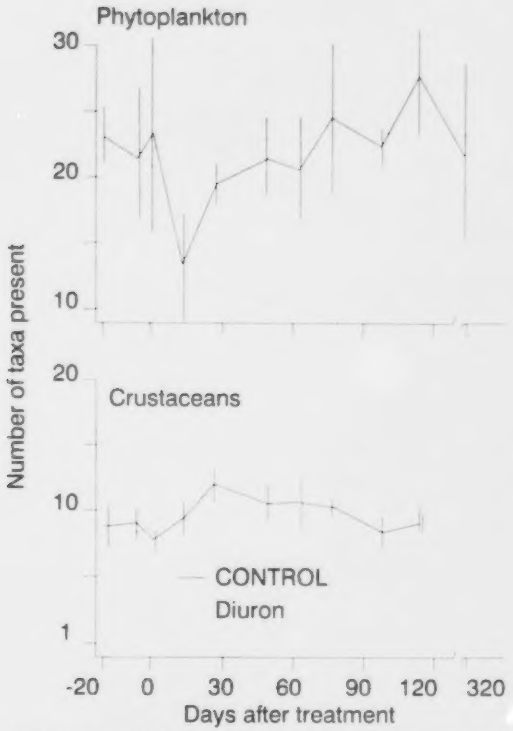

Fig. 8. The effect of diuron applied as a surface application at a concentration of 500 μg·L^{-1} on the number of taxa of phytoplankton and crustaceans present in 125-m^3 enclosures in 1982. The points represent means of three enclosures and the vertical lines indicate one standard deviation.

diuron and atrazine is the exception that tests the rule and points to the vulnerability of organisms that may be dependent on a narrow range of food sources.

Interactions

Interactions caused by selective toxicity were most clearly illustrated in the case of rotifers in the presence of permethrin. Numbers actually increased shortly after treatment at a concentration of 5 μg·L^{-1} and some time after treatment at 50 μg·L^{-1} (Kaushik et al. 1985). That the increase at 5 μg·L^{-1} was of short duration is probably due to the recovery of the larger zooplankton and is probably a result of competition for food rather than direct predation (Kaushik et al. 1985).

Route of application

Because of a lack of experience in treating enclosures with pesticides, initial experiments with permethrin used forced mixing of the pesticide with the water in the enclosure (Solomon et al. 1985). Recognizing that such mixing would be the exception in the field, where spray drift would most probably be the route of contamination, surface applications were tried with methoxychlor (Solomon et al. 1986). Surprisingly, mixing in the water column after a surface application was rapid and the pesticide was distributed evenly in the enclosure within 24–48 h of being applied to the surface.

The route of application appeared to have little impact on toxicity (as observed with methoxychlor, for which both application systems were used), but it did have a significant effect on the distribution of pesticide residues in the enclosure. Analysis of residues in water, sediments, and plastic strips of liner material (Solomon et al. 1986) showed much higher adsorption of methoxychlor to sediments in the force-mixed limnocorrals than in those that received surface treatments only. The slight disturbance of the sediments in the mixed corrals may have greatly increased the amount of particulate matter in suspension and offered the opportunity for greater adsorption and subsequent precipitation after the pump was shut down.

Results from the surface application studies suggest that the sediments act as a relatively unimportant sink in this type of system for pesticides such as methoxychlor. That the sides represent a significant sink for pesticide is important if this system is to be used to measure the distribution and fate of pesticide residues in aquatic systems. The use of limnocorrals as model validation tools will obviously require that this artificial sink be taken into consideration. Atrazine and diuron are both of relatively highly soluble in water, have low partition coefficients (compared with methoxychlor and permethrin), and show low adsorption to both the plastic sides of the corrals and the sediments in the lake. In total, these compartments represented <1% of the pesticide sink and are, therefore, considered of lesser importance in determining budgets for these and similar compounds. Other workers (Franco et al. 1984; Giddings et al. 1984; Larsen et al. 1986) have used surface applications of oil and atrazine.

Frequency of application

In this study, the biological effects of two treatments of methoxychlor at 10 $\mu g \cdot L^{-1}$ applied 35 d apart were compared with a single application at 20 $\mu g \cdot L^{-1}$.

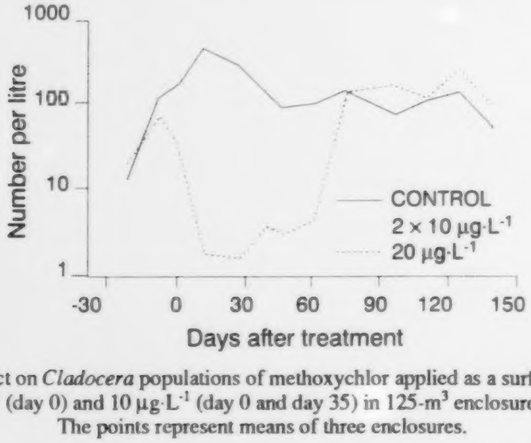

Fig. 9. The effect on *Cladocera* populations of methoxychlor applied as a surface application at 20 $\mu g \cdot L^{-1}$ (day 0) and 10 $\mu g \cdot L^{-1}$ (day 0 and day 35) in 125-m^3 enclosures in 1983. The points represent means of three enclosures.

The effect of the first installment of the double application on *Cladocera* numbers (Fig. 9) was a somewhat lower initial decline in numbers after treatment at 10 μg·L^{-1} than after treatment at 20 μg·L^{-1}. The second application of 10 μg·L^{-1} coincided with the start of recovery at both concentrations and seemed to have little or no effect on the numbers or the rate of recovery of the zooplankton. The apparent lack of toxicity to zooplankton after the second treatment was unexpected in view of the marked response after the first treatment and the previous observation of significant toxic effects at concentrations as low as 5 μg·L^{-1} (Stephenson et al. 1986). This suggests either that numbers were too low to detect a toxic response or that the organisms in the enclosure developed a tolerance or had possibly been selected for resistance to methoxychlor.

The concentrations of methoxychlor in the enclosures (Fig. 10) were as predicted and were not suggestive of changes in the distribution of the pesticide or its rate of degradation after the second treatment. These observations supported the conclusion that the absence of a further effect after the second treatment with methoxychlor was a result of changes in the biota in the system.

Effect of size

The effects of surface applications of methoxychlor (20 μg·L^{-1}) were studied in three sizes of corral. Toxicity to *Cladocera* and other sensitive zooplankton was lowest and the recovery time most rapid in the small (20-m^3) enclosures, of medium duration in the medium-sized (125-m^3) enclosures, and of the longest duration in both the edge and centre zones of the 1 000-m^3 enclosures (Fig. 11).

This size-related effect was almost certainly caused by the influence of adsorption of this pesticide to the walls of the enclosure. Small enclosures had the highest surface-to-volume ratio and dissipation of methoxychlor from the water column was most rapid (Fig. 12). Large enclosures had the lowest surface-to-volume ratio and the rate of dissipation of methoxychlor was also the lowest (Fig. 12). This observation again points to one of the major drawbacks of using enclosures to

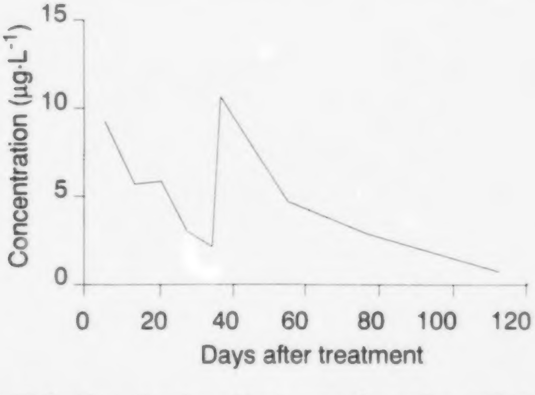

Fig. 10. Residues of methoxychlor in water after surface application of methoxychlor at 10 μg·L^{-1} on days 0 and 35. The points represent means of three enclosures.

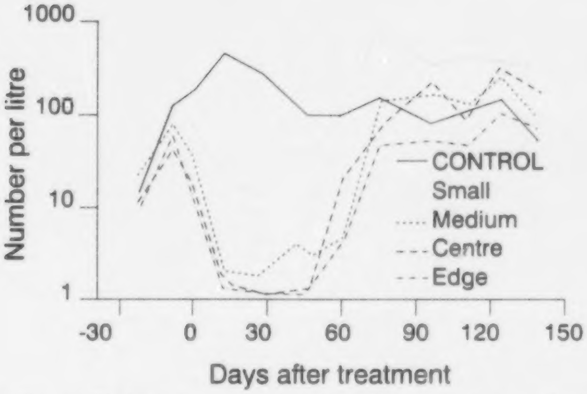

Fig. 11. The effect on *Cladocera* numbers of methoxychlor applied as a surface application at 20 $\mu g \cdot L^{-1}$ in a $20\text{-}m^3$ (small) corral, a $125\text{-}m^3$ (medium) corral, and in the edge and centre zones of a 1 $000\text{-}m^3$ corral in 1983. The points represent means of three enclosures.

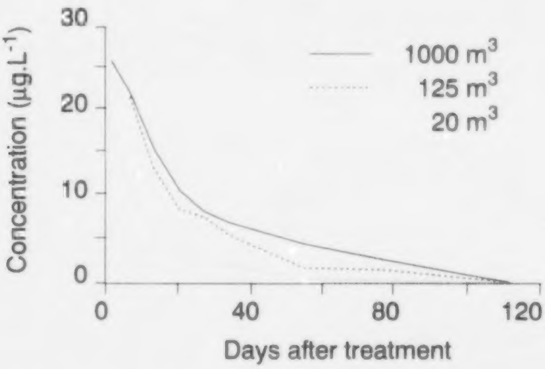

Fig. 12. Methoxychlor concentrations in water after surface application of methoxychlor at 20 $\mu g \cdot L^{-1}$ in $20\text{-}m^3$ (small), $125\text{-}m^3$ (medium), and 1 $000\text{-}m^3$ (large) corrals in 1983. The points represent means of three enclosures.

assess the effects of pesticides (or other chemicals) that tend to adsorb to the walls of the enclosure. This also suggests that impact, as measured in any size of enclosure, is likely to be less extreme than that measured in the lake or body of water in which the enclosure is located.

Taking the logistics of construction and sampling (Stephenson et al. 1984) into account, it is prudent to use as large an enclosure as possible if results are to be used to predict effects in a large body of water. Although this phenomenon is not as significant with pesticides and other compounds that do not adsorb to plastic, the wall of the enclosure is another possible source of artifact. The wall serves as a site for growth of periphyton as well as the associated community that develops in response to this habitat. This new community increases the ratio of sessile to pelagic organisms and is the probable source of much of the changes in community structure observed in enclosures maintained for long periods of time.

Community indicators

Differences in biomass rather than numbers may also indicate subtle shifts in community structure. Changes in pelagic cryptophyta biomass were much more consistent and noticeable than changes in the numbers of organisms in corrals treated with diuron at a concentration of 500 µg·L^{-1} (Fig. 13). This is, in part, an artifact of the grouping of numbers into larger taxa, which obscures the impact on individual species or the replacement of one species by another smaller organism in the same taxon. In the case of the periphyton, the effect of two treatments of atrazine at a concentration of 100 µg·L^{-1} on green algal biomass (calculated by volume) was not as obvious as its effect on the blue–green periphyton (Herman et al. 1986). However, further examination of the contribution of individual species of green algae to the total biomass showed major differences, particularly in the case of *Bulbochaeta* sp. and *Coleochaete* sp.

Although atrazine caused changes in periphytic biomass, these were not exactly paralleled by changes in chlorophyll *a* levels in the periphyton (Herman et al. 1986). Atrazine at 200 and 2 000 µg·L^{-1} caused a short-term decrease in oxygen concentration in the water column (Fig. 14), followed by an increase to a stable (but lower than in the reference enclosure) level some 60 d after treatment. This occurred despite the fact that more than 80% of the applied atrazine was still present in the water column at this time.

Atrazine (and diuron) are inhibitors of photosynthesis and, because of their persistence, were expected to cause long-term decreases in photosynthesis and oxygen

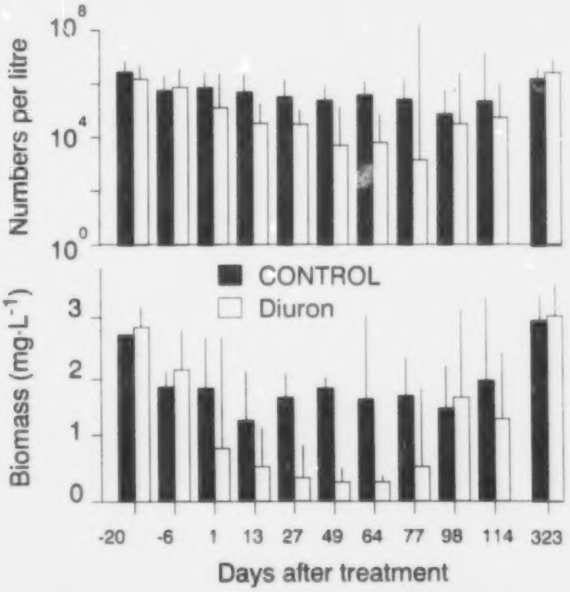

Fig. 13. The effect on numbers and biomass of cryptophyta of diuron applied as a surface application at 500 µg·L^{-1} in 125-m^3 enclosures in 1983. The points represent means of three enclosures and the vertical lines indicated one standard deviation.

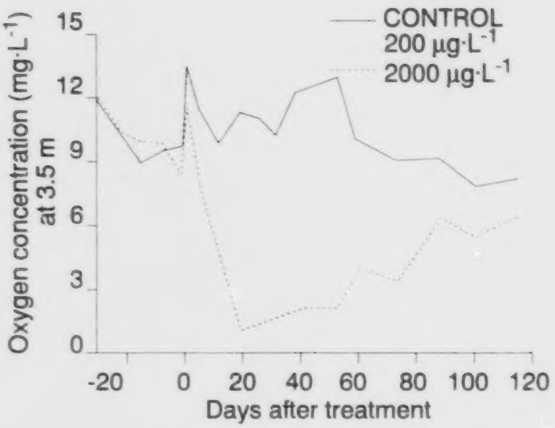

Fig. 14. The effect on oxygen concentration of atrazine applied at 2 000 and 200 $\mu g \cdot L^{-1}$ with mixing in 125-m^3 enclosures in 1981. The points represent means of three enclosures.

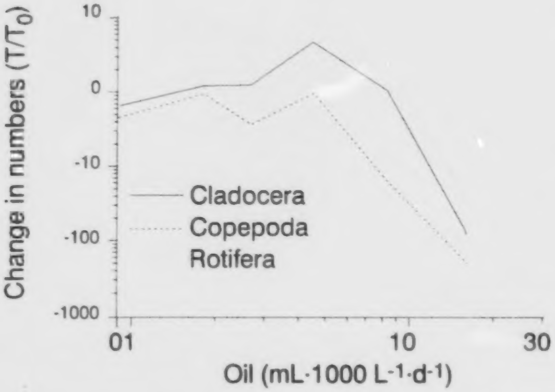

Fig. 15. The effect on *Cladocera* numbers of adding coal-derived oil to experimental ponds 4 weeks after the initiation of oiling. (Redrawn from data of Giddings et al. 1984.)

evolution. That this did not occur was due, at least in part, to an increase in the numbers of algae that were apparently less susceptible to the effects of atrazine. The reasons for this lack of sensitivity were uncertain, but may be due to an inherent lack of sensitivity, such as has been reported in terrestrial plants; increases in detoxification rates; or possible compensations in the photosynthetic process. Selection of less sensitive strains within species already present is also an interesting possibility. Differences in $^{14}CO_2$ assimilation in the periphyton (measured in situ) after two treatments with atrazine at 100 $\mu g \cdot L^{-1}$ (Herman et al. 1986) seem to confirm this suggestion. Studies by Larsen et al. (1986) on the effects of atrazine in laboratory microcosms and field mesocosms also demonstrated changes [illegible] photosynthetic activity that appeared to be related to changes in chlorophyll *a* levels.

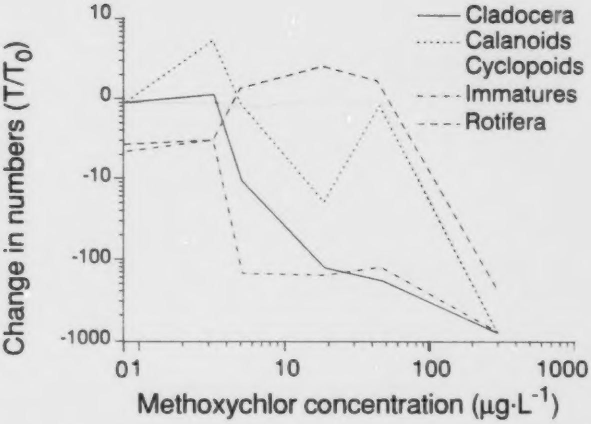

Fig. 16. The effect of methoxychlor on zooplankton numbers in treated corrals. The change in numbers represents the maximum difference between pre- and posttreatment measurements. Each output represents the mean of three enclosures. Immatures are juvenile crustacea that could not be identified.

Assessment of impact

In terms of impact assessment, the enclosure system revealed statistically significant differences in response between single species or taxa in a set of treated enclosures compared with that measured in a set of untreated reference enclosures. These differences were more obvious for those compounds that caused acute toxicity and diminished as numbers of the target species recovered to levels comparable with those in reference enclosures. Differences at the community level were less obvious and more difficult to demonstrate as one moved from individual species to larger and larger taxa. One species often increased in numbers while another, susceptible to the chemical in question, decreased.

Ascending up the scale of generalization to the level of the ecosystem, it again became progressively more difficult to observe changes due to the action of a pesticide. Gross community parameters, such as productivity, seemed to be the most insensitive to perturbation. Significant changes in individual species of phytoplankton appeared to have less overall effect on production or photosynthesis and zooplankton dependent on these primary producers seemed to be able to adjust their diet and, for the most part, did not appear to respond to a toxicant selective for phytoplankton.

In the final analysis, it is a question of defining what is meant by a significant change in an ecosystem. When is the system so different from the norm (if this can, in fact, be defined) that regulatory or preventive measures need be taken?

Ecosystem dose–response

One possible approach to the problem of assessment involves the use of the dose–response relationship. Using several concentrations of toxicant and presenting the effects graphically as a dose–response relationship allows one to interpolate pos-

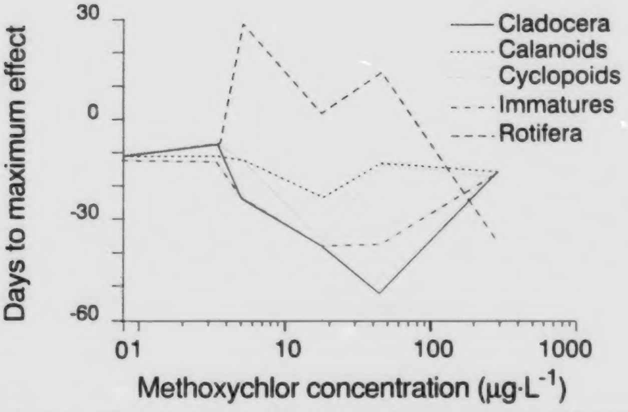

Fig. 17. Days to reach the maximum change in numbers of organisms after treatment of corrals with methoxychlor. Positive numbers indicate an increase and negative numbers a decrease in the number of organisms. Each point represents the mean of three enclosures. Immatures are juvenile crustacea that could not be identified.

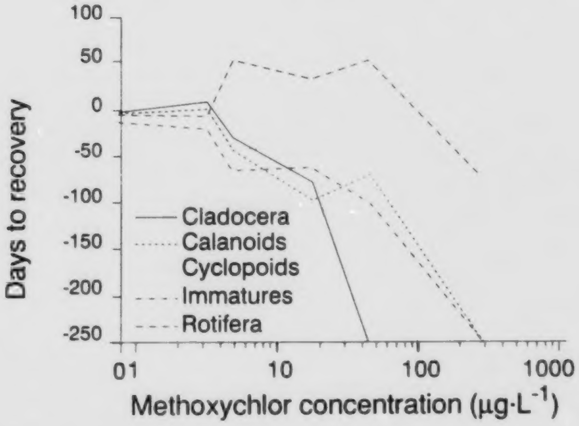

Fig. 18. Days to population recovery after treatment of corrals with methoxychlor. Positive numbers indicate an increase and negative numbers a decrease in the number of organisms. Each point represents the mean of three enclosures. Recovery was defined as the point at which numbers of organisms in the treated enclosures were within 20% of those in the reference enclosures. Immatures are juvenile crustacea that could not be identified.

sible effects at concentrations other than those used and, as such, presents an ideal regulatory tool.

Analysis of dose–response data has been carried out by a number of authors. Giddings et al. (1984), working on oil, showed responses suggesting that a plateau of no response existed at rates of addition of <4 $mL \cdot m^{-3} \cdot d^{-1}$ (Fig. 15). The work of Larsen et al. (1986) similarly demonstrated dose–response relationships to atrazine in community parameters, such as photosynthesis, at several times after treatment.

The work conducted in this study on methoxychlor used several concentrations and dose–response relationships were also observed.

In terms of mortality, or changes in numbers, an increase in the numbers of *Cladocera* was noted at the lowest concentration of 3 $\mu g \cdot L^{-1}$, whereas at higher concentrations, significant decreases occurred. Similar trends were observed for other organisms, with rotifers showing the lowest sensitivity and, in fact, increasing in numbers at concentrations between 5 and 50 $\mu g \cdot L^{-1}$ (Fig. 16). Because the present studies involved single applications only, dose–response relationships could be observed in both the time to effect and the time to recovery, potentially important endpoints from a regulatory point of view.

In terms of the time to effect, response times showed a dose–response relationship at concentrations between 5 and 50 $\mu g \cdot L^{-1}$ (Fig. 17); however, response time was much shorter at 300 $\mu g \cdot L^{-1}$, suggesting the greater significance of acute response at this level of exposure. In terms of recovery time, similar dose–response relationships were observed, although the *Cladocera* appeared to be the most sensitive (Fig. 18).

The concept of ecosystem dose–response certainly appears to hold considerable promise; however, the most important question, that of the overall significance of these changes, is seldom addressed and remains essentially unanswered.

Acknowledgments

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Baker, D.B. 1983. Studies of sediment, nutrient and pesticide loading in selected Lake Erie and Lake Ontario tributaries: Part IV — Pesticide concentrations and loading in selected Lake Erie tributaries, 1982 — Final draft report.United States Environmental Protection Agency, Washington, DC, USA. USEPA Grant R005708-01 (1982).

Beers, J.R., Reeve, M.R., Grice, G.D. 1977. Controlled ecosystem pollution experiment: Effects of mercury on enclosed water columns: IV — Zooplankton population dynamics and production. Marine Science Communications, 3, 355–394.

Beyers, R.T. 1962. The metabolism of twelve aquatic laboratory microecosystems. Ecological Monographs, 33, 281–306.

Blinn, D.W., Tompkins, T., Zaleski, L. 1977. Mercury inhibition of primary productivity using large volume plastic enclosures in situ. Journal of Phycology, 13, 58–61.

Butler, P.A. 1963. Pesticide–wildlife studies: a review of Fish and Wildlife Service investigations during 1961 and 1962. United States Fish and Wildlife Service, Washington, DC, USA. Circular 167.

Davies, J.M., Gamble, J.C. 1979. Experiments with large enclosed ecosystems. Philosophical Transactions of the Royal Society of London (Series B: Biological Sciences), 286, 523–544.

Franco, P.J., Giddings, J.M., Herbes, S.E., Hook, L.A., Newbold, J.D., Roy, W.K., Southworth, G.R., Stewart, A.J. 1984. Effects of chronic exposure to coal-derived oil on freshwater ecosystems: 1 — Microcosms. Environmental Toxicology and Chemistry, 3, 447–463.

Giddings, J.M., Franco, P.J., Cushman, R.M., Hook, L.A., Southworth, G.R., Stewart, A.J. 1984. Effects of chronic exposure to coal-derived oil on freshwater ecosystems: II — Experimental ponds. Environmental Toxicology and Chemistry, 3, 465–488.

Goldman, C.R. 1962. A method for studying nutrient limiting factors in situ in water columns isolated by polyethylene film. Limnology and Oceanography, 7, 99–101.

Herman, D., Kaushik, N.K., Solomon, K.R. 1986. Impact of atrazine on periphyton in freshwater enclosures and some ecological consequences. Canadian Journal of Fisheries and Aquatic Sciences, 43, 1 917–1 925.

Kaushik, N.K., Stephenson, G.L., Solomon, K.R., Day, K.E. 1985. Impact of permethrin on zooplankton communities using limnocorrals. Canadian Journal of Fisheries and Aquatic Sciences, 42, 77–85.

Kaushik, N.K., Solomon, K.R., Stephenson, G.L., Day, K.E. 1986. Use of limnocorrals in evaluating the effects of pesticides on zooplankton communities. *In* Cairns, J., Jr. ed., Community toxicity testing. American Society for Testing and Materials, Philadelphia, PA, USA. STP 920, 269–200.

Kuiper, J. 1981. Fate and effects of mercury in marine plankton communities in experimental enclosures. Ecotoxicology and Environmental Safety, 5, 106–134.

Lack, T.J., Lund, W.G. 1974. Observations and experiments on the phytoplankton of Bellham Tarn, English Lake District. Freshwater Biology, 4, 399–415.

Larsen, D.P., DeNoyelles, F., Jr., Stay, F., Shiroyama, T. 1986. Comparisons of single-species, microcosm and experimental pond responses to atrazine exposure. Environmental Toxicology and Chemistry, 5, 179–190.

Leah, R.T., Moss, B., Forrest, D.E. 1978. Experiments with large enclosures in a fertile, shallow, brackish lake, Hickling Broad, Norfolk, United Kingdom. Internationale Revue der Gesamten Hydrobiologie, 63, 291–310.

Marshall, J.S., Mellinger, D.L. 1980. An in situ experimental method for toxicological studies on natural plankton communities. *In* Eaton, J.G., Parish, P.R., Hendricks, A.C., ed., Aquatic toxicology. American Society for Testing and Materials, Philadelphia, PA, USA. STP 707, 27–39.

Menzel, D.W., Case, J. 1977. Concept and design: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 1–7.

Shires, S.W. 1983. The use of small enclosures to assess the toxic effects of permethrin in fish under field conditions. Pesticide Science, 14, 475–480.

Solomon, K. R., Smith, K.E., Guest, G., Yoo, J., Kaushik, N.K. 1980. The use of limnocorrals in studying the effects of pesticides in the aquatic ecosystem. Canadian Technical Report on Fisheries and Aquatic Sciences, 975, 1–9.

Solomon, K.R., Yoo, J.Y., Lean, D., Kaushik, N.K., Day, K.E., Stephenson, G.L. 1985. Dissipation of permethrin in limnocorrals. Canadian Journal of Fisheries and Aquatic Sciences, 42, 70–76.

______ 1986. Methoxychlor distribution, dissipation and effects in freshwater limnocorrals. Environmental Toxicology and Chemistry, 5, 577–586.

Stephenson, G.L., Hamilton, P., Kaushik, N.K., Robinson, J.B., Solomon, K.R. 1984. The effect of enclosure size on the spatial distribution of zooplankton. Canadian Journal of Fisheries and Aquatic Sciences, 41, 1 048–1 054.

Stephenson, G.L., Kaushik, N.K., Solomon, K.R., Day, K.E., Hamilton, P. 1986. Impact of methoxychlor on freshwater plankton communities in limnocorrals. Environmental Toxicology and Chemistry, 5, 587–603.

Stratton, G.W. 1984. Effects of the herbicide atrazine and its degradation products, alone and in combination, on phototrophic microorganisms. Archives of Environmental Contamination and Toxicology, 13, 35–42.

Strickland, J.D.H., Terhune, L.D.B. 1961. The study of in situ marine photosynthesis using a large plastic bag. Limnology and Oceanography, 6, 93–96.

Torres, A.M.R., O'Flaherty, L.M. 1976. Influence of pesticides on *Chlorella, Chlorococcum, Stigeoclonium* (Chlorophyceae), *Tribonema vancheria,* (Xanthophyceae) and *Oscillatoria* (Cyanophyceae). Phycologia, 15, 25–36.

Twinch, A.J., Breen, C.M. 1981. The thermal, oxygen and light regimes in isolated columns of water in a turbid impoundment. Freshwater Biology, 10, 207–213.

Whittaker, R.H. 1961. Experiments with radiophosphorus tracer in aquarium microcosms. Ecological Monographs, 31, 157–188.

Control of Phytoplankton Blooms in the Subarctic Pacific Ocean — Experimental Studies in Microcosms

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The goal of the Subarctic Pacific Ecosystem Research (SUPER) program is to understand the biological, chemical, and physical mechanisms that prevent blooms of phytoplankton from occurring in the oceanic subarctic Pacific. Historically, bloom control has been attributed to grazing pressure by large, endemic copepods of the genus Neocalanus. *As part of the research conducted during two cruises in 1984 (May and August), the dynamics of phytoplankton community growth in 60-L microcosms incubated with and without copepod grazers and with and without an additional nitrogen source (ammonium) were explored. In May, phytoplankton abundance, measured as chlorophyll, particulates, and microscopical counts, bloomed dramatically in the absence of copepod grazers, but was controlled by* Neocalanus plumchrus *copepodids at a density of one copepod per litre. Moreover, species composition remained more stable in the presence of the copepods. Ammonium did not appear to exert a substantial stimulatory effect in May except for a greater dominance of diatoms in microcosms without copepod grazers. Despite 10* µM *nitrate in the surface water during August, phytoplankton incubated without copepods did not bloom unless ammonium was added. Thus, the August situation resembled in many respects the close coupling of phytoplankton growth and ammonium regeneration characteristic of oligotrophic systems.*

Results of microcosm experiments as well as additional experimental work from the first SUPER cruises point to microzooplankton as the important grazers of the dominant picoplankton-sized primary producers in the subarctic Pacific. Large copepods, although contributing to the grazing pressure on phyto- plankton, particularly during spring and early summer, are probably most important in modulating microzooplankton grazing by predation and in controlling larger phytoplankton forms, such as diatoms. Failure of phytoplankton to grow rapidly on the excess available nitrate in late summer suggests that some factor, presumably iron, required for the efficient use (uptake or reduction) of nitrate may be diminished during the growing season. Limited mixing through the highly stable permanent halocline in the subarctic Pacific may prevent adequate replenishment of this required factor from deeper waters during winters.

The oceanic subarctic Pacific is unique among temperate and polar open ocean systems as well as adjacent coastal waters in that it does not have dramatic seasonal blooms in diatom abundance (Parsons et al. 1966; Anderson et al. 1969, 1977). Data from the Canadian weathership Station P are representative of this region (Fig. 1). During more than 20 years of observation at Station P, phytoplankton abundance, measured as chlorophyll *a*, has remained low and monotonously constant despite seasonal stratification of the euphotic zone, high levels of dissolved inorganic nitrogen in the form of nitrate (Anderson et al. 1969), and a pronounced seasonal signal in the rate of primary production measured with ^{14}C (Parsons et al. 1966). Understanding the mechanisms that control the abundance of phytoplankton in this region is the major goal of the Subarctic Pacific Ecosystem Research (SUPER) project.

The historical explanation for the lack of phytoplankton blooms in the subarctic Pacific is that grazing by large, endemic copepods of the genus *Neocalanus*, which dominate the macrozooplankton biomass in the region, continuously crops phytoplankton to a low level (Beklemishev 1957; Heinrich 1957, 1962; McAllister et al. 1960; Parsons and LeBrasseur 1968). Indeed, these copepods have several characteristics that could contribute to grazing control: a unique life history that sends large numbers of juvenile stages to surface waters before physical conditions favour blooms (e.g., Miller et al. 1984), the ability to feed effectively on low densities of the very tiny cells that dominate phytoplankton abundance in the region (McAllister et al. 1960; Booth et al. 1982), and a stabilizing functional response to increasing phytoplankton abundance (Frost et al. 1983). However, *Neocalanus* spp undergo a seasonal migration that removes them from surface waters in late summer when physical conditions are still conducive for blooms. Consequently, at least during this period, phytoplankton blooms must be controlled by alternative mechanisms — grazing by smaller consumers (e.g., microzooplankton) or possibly the lack of an essential growth factor.

This paper presents the results of three preliminary experiments that investigate possible direct (grazing) and indirect (excretion) roles of grazers in suppressing

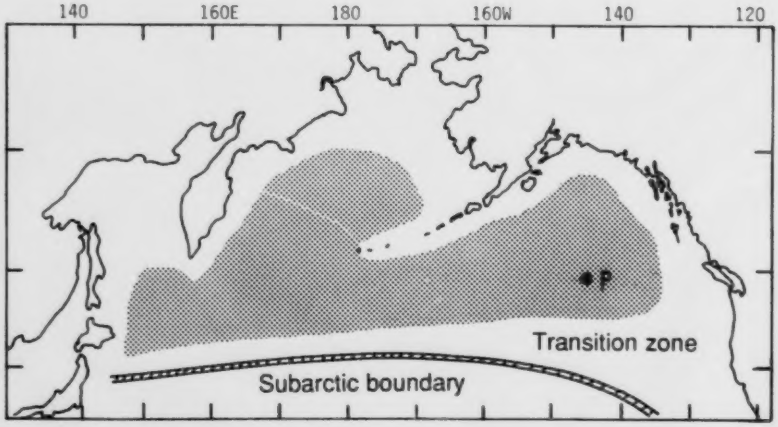

Fig. 1. Areal extent of the subarctic Pacific showing the location of Canadian weathership Station P.

diatom blooms in the subarctic Pacific. Specifically, shipboard experiments in enclosures were used to address two questions:

- First, can grazing pressure by the dominant macrozooplankter, *Neocalanus plumchrus*, control phytoplankton abundance under seminatural conditions? and
- Second, is the presence of the zooplankton excretion production, ammonium, an important growth factor for diatoms in this otherwise high-nitrate environment?

Aside from the practical problems associated with deploying and maintaining ecosystem-sized mesocosms in a high-energy oceanic environment, there was reason to believe that meaningful answers to these questions could be obtained in modest-sized microcosms incubated on shipboard. It was not necessary, for instance, to consider the role of parameters that varied vertically in the water column because the phenomenon of interest was confined to a relatively thin (20–30 m), light-saturated, upper mixed layer with little communication with underlying waters (K. Denman and A. Gargett, unpublished data).

The experimental animals, late copepodid stages of *Neocalanus plumchrus*, are dominant and almost exclusively distributed in the mixed layer during late spring and early summer and do not complicate experimental design and interpretation with diurnal migratory behaviour or reproduction. In addition, *Neocalanus* copepodids, despite their large size, are relatively inactive and, therefore, are not unduly stressed in modest-sized microcosms. More importantly, because the stability of the phytoplankton community of the subarctic Pacific is a long-term observation applicable over a wide range of environmental conditions, the purpose of the exercise was not to follow the dynamics of the system over the course of generations of consumers, a typical motivation for mesocosm-scaled experiments (Grice and Reeve 1982), but to test the extent to which different treatments move the community away from its equilibrium position. Thus, experiments were designed on a time scale of several days to a week.

Methods

Temporal changes in the abundance and composition of natural particulates were observed in 60-L microcosms made from polyethylene drum liners (J.F. Shelton Co.). The microcosm drums were closed with a screw-top cap after filling and sampled through a silicone umbilical cord connecting through the cap to a rigid tube with a flared outlet at the centre of the drum. The outlet was covered with 500-µm Nitex screening to avoid removal of macrozooplankton. The silicone tubes had stopcocks near the sampling end to completely close the containers when they were not being sampled. Before the cruise, all microcosm containers, tubes, etc. were thoroughly cleaned following the method of Fitzwater et al. (1982).

Shipboard experiments were conducted in a four-drum incubation system during cruises in May and August 1984 in the vicinity of Station P (50°N, 145°W). The containers were gently filled with prescreened (200-µm) surface seawater collected before sunrise, i.e., after the night's thermal overturn of the mixed layer (upper 20–40 m). Different treatments involved additions of copepodid C5s of *Neocalanus plumchrus*, NH_4, or both. Ammonium enrichments were added to the containers as

NH_4Cl when they were about half full to facilitate complete mixing. *Neocalanus* copepodids were gently collected from short vertical hauls (20 m to surface) using a 0.5-m, 300-µm mesh plankton net with a large (15-L) codend. Active copepods were sorted from the net codend, washed with fresh surface water, and added to the microcosms when filling was near completion. After filling, the lids were closed and the umbilical cord flushed to exclude all air bubbles. Each container was placed in a bag of neutral density fabric to decrease the light level to about 40% of surface irradiance, about the ambient light level at 10 m. The four drums were then incubated parallel to one another while they were rotated slowly (about 0.5 rpm) on their horizontal axes in a large Plexiglas bath cooled with seawater from about 5 m (about 7°C in May and 12°C in August 1984). The rotating mechanism consisted of stainless steel frames (to hold the containers) geared to a positive chain drive. Power was provided from the pressure of inflow cooling water striking a paddle flywheel. Cooling volume and rotation speed were adjusted independently.

The containers were sampled each morning by drawing water through the silicone umbilical tube to a closed flask under slight negative pressure from a hand-held vacuum pump. A volume several times that of the silicone and Plexiglas sampling tube was discarded before samples were drawn for chlorophyll, nutrient, particle volume, and phytoplankton cell counts. The walls of the drum liner collapsed to accommodate the volume of water removed by subsampling. Unbiased sampling through the umbilical tube was confirmed by comparing results from routine water samples taken on the last day of each experiment with samples taken shortly thereafter as the microcosms were emptied after vigorous agitation.

Phytoplankton pigments were extracted in 90% acetone and analyzed fluorometrically (Lorenzen 1966). Nutrient concentrations (nitrate, nitrite, ammonium, phosphate, and silicate) were determined from the freshly collected samples using standard autoanalyzer techniques. Particulate abundances and biovolume distributions for 128 size classes of particulates in the range of 2–40 µm equivalent spherical diameter were counted with an Elzone 80XY particle analyzer (Particle Data, Inc.). Samples for microscopical analysis were preserved with 1% gluteraldehyde and refrigerated in the dark until slides were prepared, up to 4 weeks after collection, using primulin (Caron 1983) and DAPI (Porter and Feig 1980) staining techniques. Bacterial, flagellate, and diatom populations were enumerated by epifluorescence microscopy.

Results

Copepod grazing effects

One of the microcosm experiments conducted during the May 1984 cruise was designed to test the ability of *Neocalanus plumchrus* C5 copepodids to suppress phytoplankton blooms. Copepods were added to two of four containers at a density of one copepod per litre. Ammonium (4.5 µ*M*) was also added to one of the containers with copepods and one without copepods to distinguish between copepod grazing and excretion effects. All of the copepods were recovered alive at the end of the experiment.

The presence of the large grazing copepods suppressed phytoplankton blooms in treatments with or without added ammonium. Phytoplankton abundance, measured as chlorophyll and particle counts, increased markedly in the absence of *Neocalanus*, but not when they were present (Fig. 2). Ammonium, when available, was utilized preferentially to nitrate (Fig. 3). However, in the control treatment without added ammonium or copepods, the bloom resulted in a substantial depression of nitrate levels (2 μ*M*) over the course of the week. Both diatoms and autotrophic nanoplankton (microflagellates and coccoids) contributed to the blooms in the absence of grazers (Fig. 4), whereas species composition of the phytoplankton

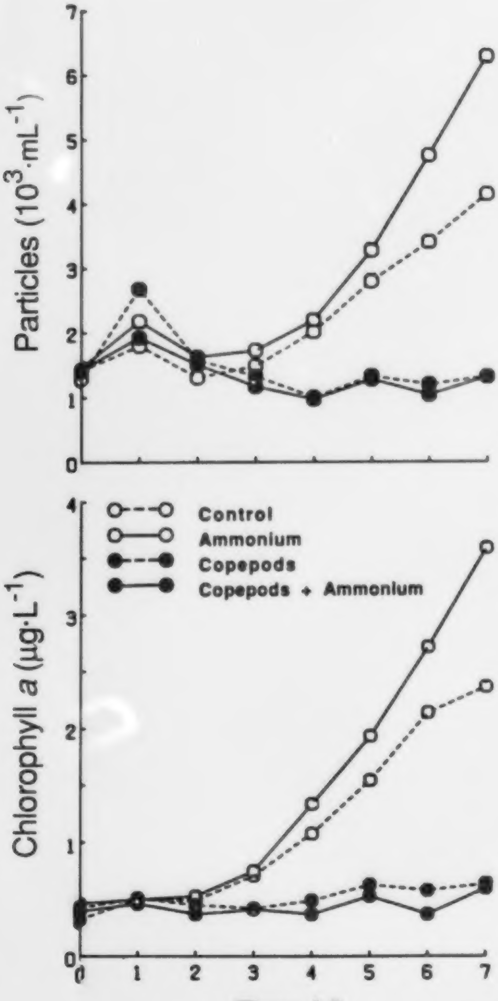

Fig. 2. Effects of the presence of copepods (*Neocalanus plumchrus* C5 — one per litre) and ammonium (4.5 μ*M*) on phytoplankton abundance, measured as total particle density and chlorophyll *a*, during the 11–18 May 1984 microcosm experiment in the subarctic Pacific.

assemblage remained more stable with *Neocalanus* present. In particular, the cyanobacteria *Synechoccoccus* rapidly disappeared in containers without copepods. This is attributed to a secondary food-web effect, i.e., the copepods controlled abundance levels of zooflagellates, the dominant consumers of cyanobacteria. Attempts were made to conduct a follow-up experiment using lower copepod densities (one copepod per 3 L). However, the experiment was cut short (the cruise ended) just as phytoplankton abundance in control microcosms began to diverge (i.e., bloom) from experimental containers.

Ammonium effects

Microcosm experiments were continued during the August 1984 cruise after *Neocalanus plumchrus* had migrated out of the euphotic zone. In the first of two experiments, replicate microcosms were prepared with and without a high

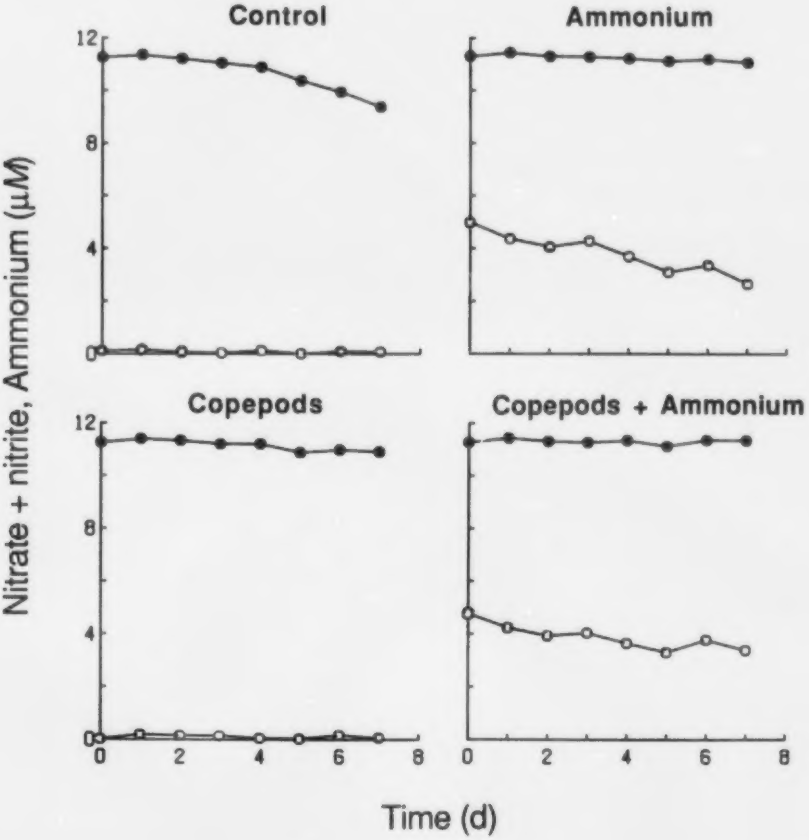

Fig. 3. Disappearances of nitrate–nitrite (closed circles) and ammonium (open circles) during the 11–18 May 1984 microcosm experiment.

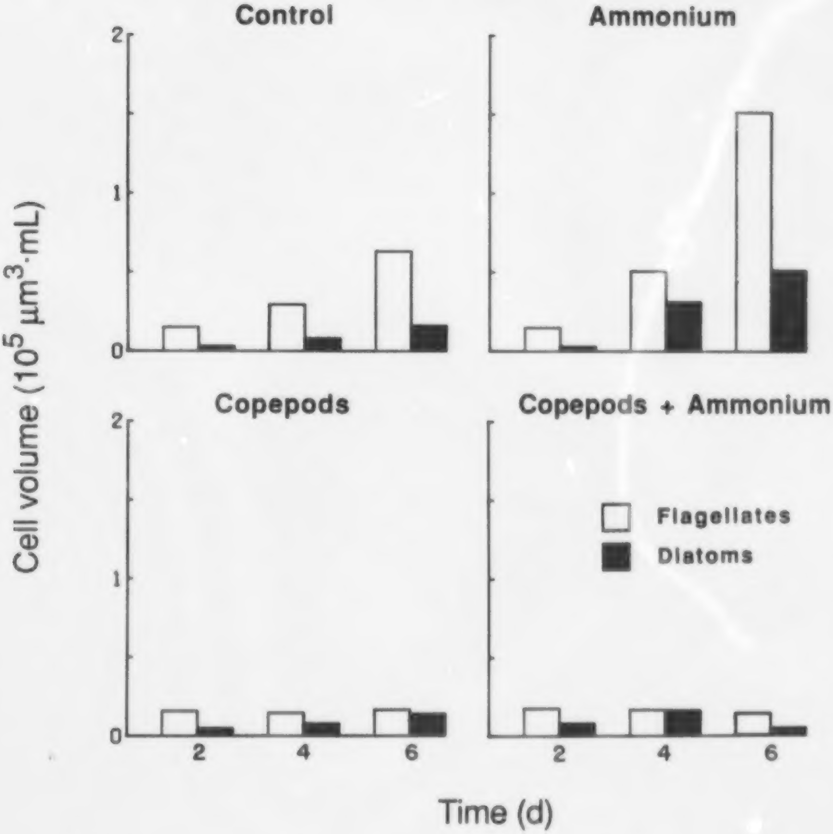

Fig. 4. Effects of zooplankton grazing and ammonium addition on the composition of the phytoplankton community (11–18 May 1984).

ammonium addition (4.5 µ*M*). In the second experiment, a range of ammonium additions was prepared 0, 0.5, 1.0, and 2.5 µ*M*. Because of rapid utilization of the added ammonium during this later experiment, treatments were respiked with ammonium after 2 days. Natural background levels of ammonium were 0.1–0.2 µ*M* during August.

A dramatic difference in the growth response of phytoplankton was observed in the first experiment with 4.5 µ*M* ammonium as opposed to controls without ammonium. Total suspended particulates and chlorophyll *a* increased substantially in microcosms with added ammonium but not in those without ammonium (Fig. 5), although the viability and growth of phytoplankton in microcosms without ammonium was evident from declining nitrate concentrations (Fig. 6). This result implies that phytoplankton abundance is controlled under ambient growth conditions by grazers in the microzooplankton community. In contrast, the elevated ammonium concentrations stimulated growth of both microflagellates and diatoms over seawater controls (Fig. 7). However, diatoms had the highest net population growth

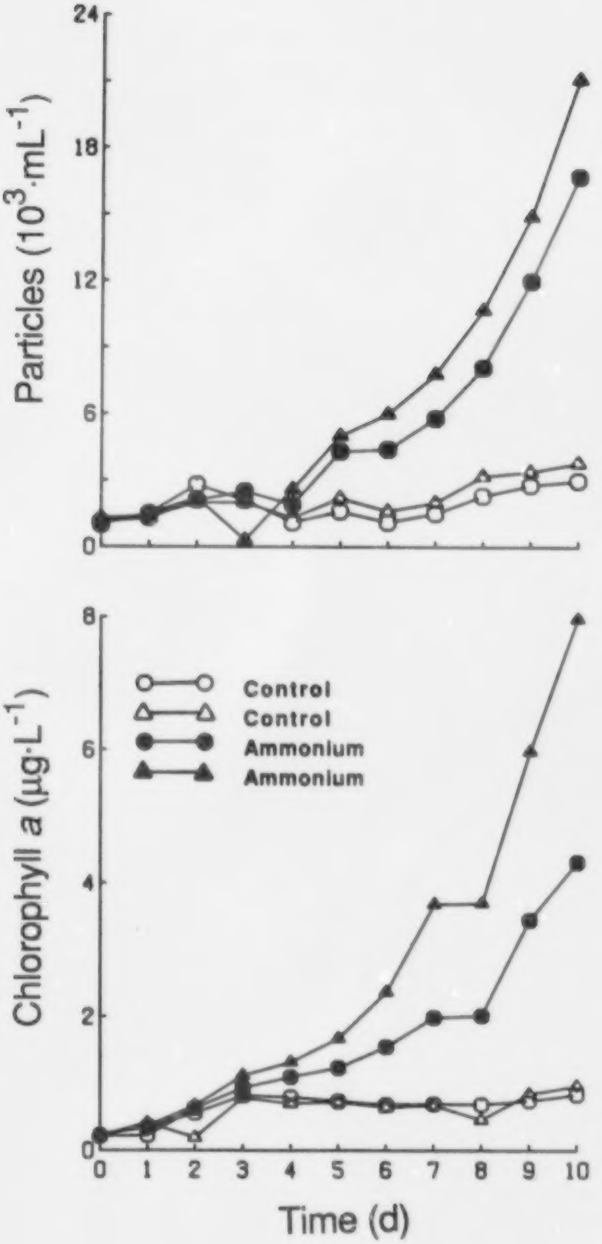

Fig. 5. Effects of ammonium (4.5 µ*M*) on phytoplankton abundance, measured as total particle density and chlorophyll *a*, during the 3–13 August 1984 microcosm experiment in the subarctic Pacific.

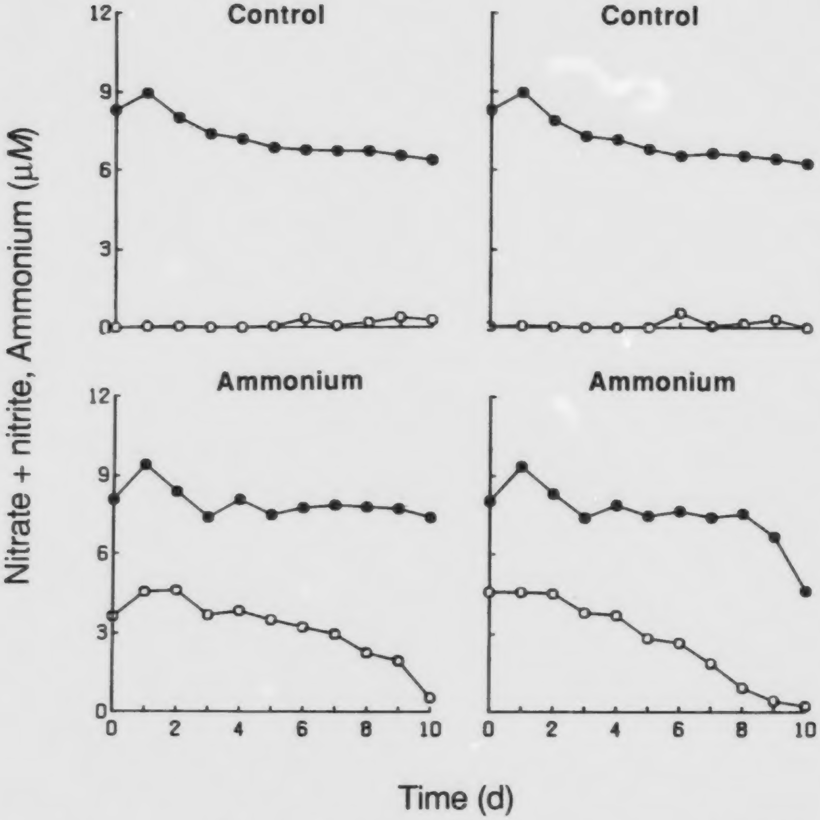

Fig. 6. Disappearance of nitrate–nitrite (closed circles) and ammonium (open circles) during the 3–13 August 1984 microcosm experiment.

response to added ammonium because ammonium shifted the composition of the diatom community from larger, relatively rare species to smaller, more abundant chain-forming pennates.

In the final microcosm experiment, total phytoplankton biomass (total counts of suspended particles, chlorophyll, and cell abundance) increased in all microcosms, but the net increase varied positively with increasing concentrations of ammonium added (Fig. 8). The increased phytoplankton biomass came at the expense of ammonium utilization as the disappearance of nitrate was comparable for all treatments (Fig. 9). Both autotrophic nanoflagellates and diatoms increased in cell abundance at all levels of ammonium added (Fig. 10). Nanoflagellate populations (cell numbers) grew approximately equally at the different ammonium concentrations, although larger forms were more prevalent at high concentration of ammonium (data not shown). The differences in the total phytoplankton growth responses among ammonium treatment levels were largely due to diatoms that grew faster at higher concentrations of ammonium.

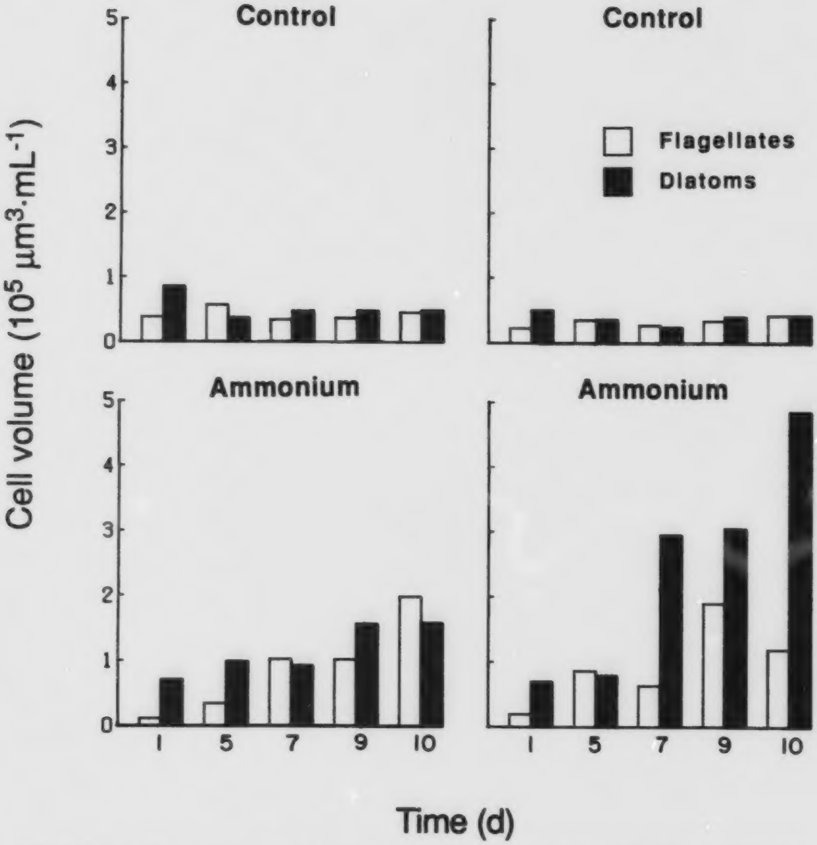

Fig. 7. Effects of ammonium addition on the composition of the phytoplankton community (3–13 August 1984).

Discussion

Results of preliminary shipboard microcosm experiments conducted during the first phase of the SUPER program suggest that both direct (grazing) and indirect (excretion) effects of consumer populations may be important in controlling phytoplankton growth and abundance in the oceanic subarctic Pacific. The copepod *Neocalanus plumchrus* contributes to the control of phytoplankton abundance and community composition when it is present during the spring.

Observed grazing control in the microcosms implied mean clearance rates of about 350 mL·d^{-1} per copepod to balance net phytoplankton growth rates in microcosms without copepods. Rates on this order have previously been measured in shipboard and laboratory experiments (Frost et al. 1983; Dagg and Walser 1987). Nonetheless, at observed densities of copepodids in the water column during the May 1984 cruise, these clearance rates would have been insufficient for

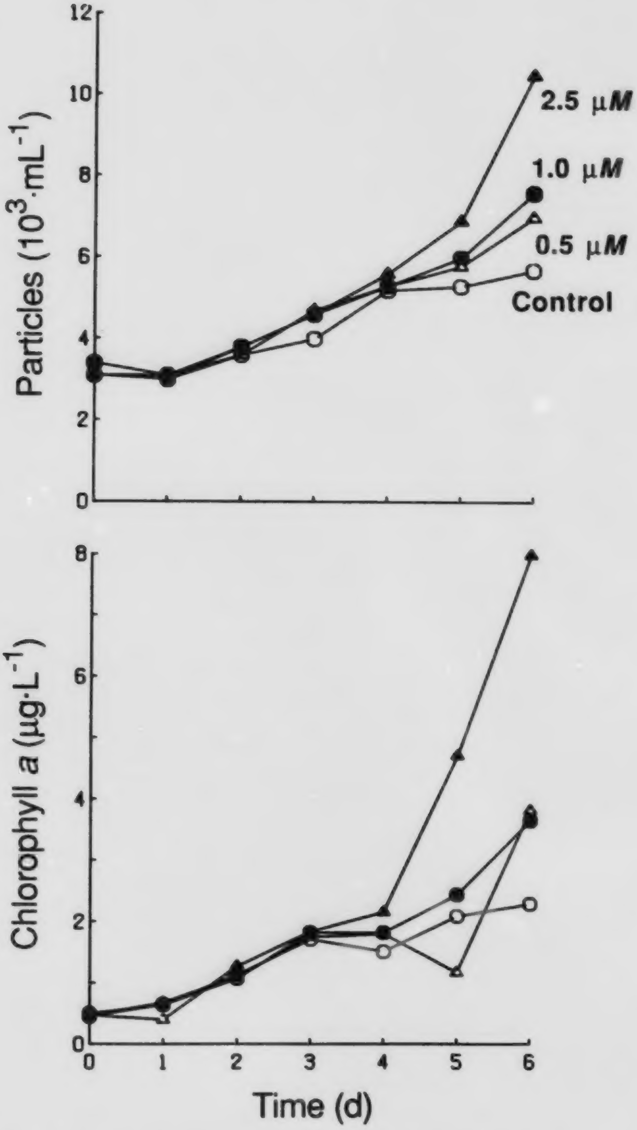

Fig. 8. Effects of ammonium concentration on phytoplankton abundance, measured as total particle density and chlorophyll *a*, during the 20–26 August 1984 microcosm experiment in the subarctic Pacific.

Neocalanus to account for a major component of grazing. In addition, various indirect lines of evidence, including pigment budgets (after Welschmeyer and Lorenzen 1985), dilution experiments (after Landry et al. 1984), and simulation modeling (Frost 1987), have pointed to the importance of microzooplankton as dominant

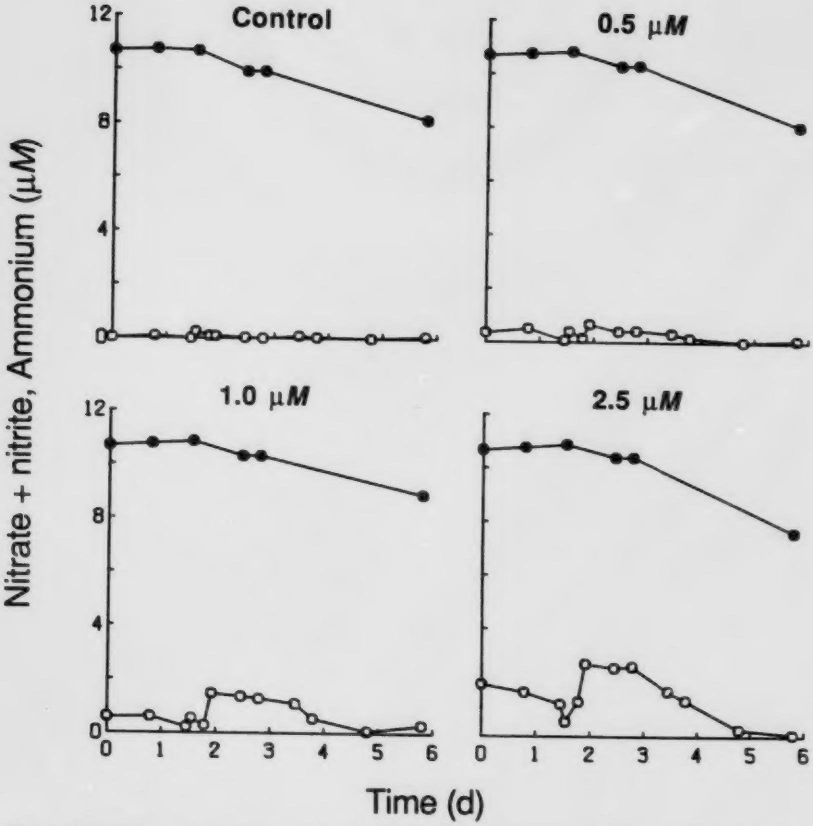

Fig. 9. Disappearance of nitrate–nitrite (closed circles) and ammonium (open circles) during the 20–26 August 1984 microcosm experiment.

consumers of phytoplankton production. However, omnivorous suspension feeding by *Neocalanus* species may play a pivotal role in controlling populations of diatom cells and chains too large to be grazed directly by microzooplankton as well as in controlling microzooplankton populations (Frost 1987). Further studies are required, using lower densities of grazers in experimental microcosms, to determine potential grazing control by *Neocalanus* spp.

A second significant result from these microcosm experiments was that plankton growth could always be stimulated above ambient levels by adding ammonium despite consistently high levels of dissolved nitrate. In independent experiments on the same cruises, P. Wheeler (personal communication) observed greatly reduced rates of nitrate uptake in August (20–40 mg N·m^{-3}·d^{-1}) relative to May (100–180 mg N·m^{-3}·d^{-1}), suggesting that phytoplankton production during summer may be tied to recycled ammonium despite remaining high concentrations of nitrate in surface waters. This could be particularly true for diatoms, which at low levels of ammonium presumably have a competitive disadvantage in nutrient uptake relative to nanoflagellates.

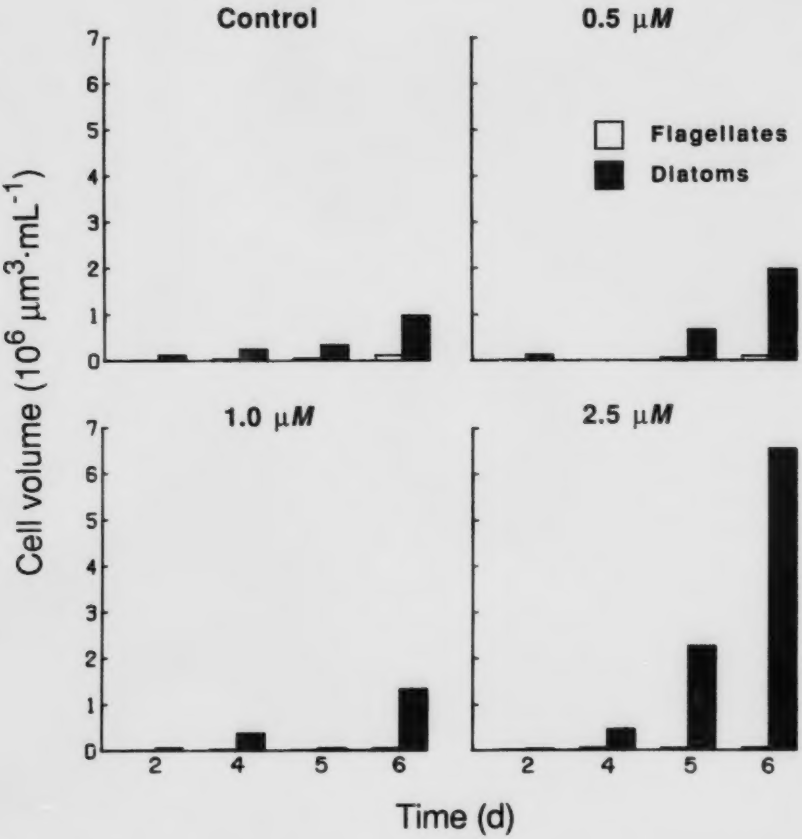

Fig. 10. Effects of ammonium concentration on the composition of the phytoplankton community (20–26 August 1984).

These results imply that nitrate uptake or utilization (reduction) may be limited by a trace factor, presumably iron, as suggested from recent experimental work by Martin and Fitzwater (1988). The permanent halocline in the subarctic Pacific, which limits the introduction of deep water to the euphotic zone even during the severe weather conditions experienced in winter (e.g., Evans and Parslow 1982), is one mechanism that would perpetuate this limitation.

Conclusions

In summary, the results of this study, along with others during the first phase of the SUPER program, indicated that grazing by copepods is not sufficient to control phytoplankton abundance in the subarctic Pacific. Microcosm studies provided evidence of some control potential for *Neocalanus plumchrus*, which would be of consequence in suppressing phytoplankton blooms in the spring and early summer.

However, copepods were not abundant enough in 1984 to contribute substantially to a general grazing balance. Utilization and control of dominant photoautotrophs, cyanobacteria, and small eucaryotes (<2 μm in diameter) must generally rest with zooflagellates and ciliated protozoa because this fraction is too small to be grazed effectively by the large copepods. The mechanism suppressing diatom abundance during the summer, when there are few *Neocalanus* in the upper mixed layer, is still an open issue. Results from this study suggest that they may, in part, be limited in their ability to compete effectively for the low concentration of ammonium recycled within the microbial and nanoplankton community. This is consistent with the view that the normal pathway for using dissolved nitrate may be limited by the availability of a trace element. Alternatively, large, naked ciliates, which are more prevalent in summer than spring, presumably because they are released from predation by *Neocalanus* copepodids, may be effective consumers of diatoms.

Microcosms provide two major advantages for studying interactions among production, grazing, and nutrient-cycling processes in the open-ocean plankton of the subarctic Pacific. First, the microcosms hold a sufficient volume of water to allow low-level, long-term experiments or intensive, short-term experiments to be carried out by numerous investigators to address many related problems simultaneously. Second, the microcosms hold replicated, revisitable natural communities under known conditions of lighting, temperature, nutrients, and macrozooplankton grazing in which one can study the rates of important processes relating, for example, to the nitrogen budget and expect that their net effects on standing stocks and concentrations will be evident as a direct test of our ability to unambiguously close the budget. Moreover, potential ecosystem control points can be manipulated in the microcosms and the community response used as a measure of their influence on the balance phenomenon.

Experiments during the first phase of the SUPER program demonstrated the utility of the microcosm approach. Experiments to be conducted during the second phase of the project (beginning in May 1987) will represent a more extensive, collaborative undertaking to investigate the interactions of copepods, microzooplankton, algae, bacteria, and nutrients in an attempt to explain the unique characteristics of the subarctic Pacific ecosystem.

Acknowledgments

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Anderson, G.C., Parsons, T.R., Stephens, K. 1969. Nitrate distribution in the subarctic northwest Pacific Ocean. Deep-Sea Research, 16, 329–334.

Anderson, G.C., Lam, R.K., Booth, B.C., Glass, J.M. 1977. A description and numerical analysis of the factors affecting the processes of production in the Gulf of Alaska. Department of Oceanography, University of Washington, Seattle, WA, USA. Special report, 231 pp.

Beklemishev, K.V. 1957. The spatial relationships of marine zoo- and phytoplankton. *In* Nikitin, B.N., ed. 1959. Marine biology. American Institute of Biological Sciences, Washington, DC, USA. [Translation from Russian, original in Trudy Instituta Okeanologii, Akademiya Nauk SSSR, 20, 253–278.]

Booth, B.C., Lewin, J., Norris, R.E. 1982. Nanoplankton species predominant in the subarctic Pacific in May and June 1978. Deep-Sea Research, 29, 185–200.

Caron, D.A. 1983. A technique for the enumeration of heterotrophic and phototrophic nanoplankton using epifluorescence microscopy, and a comparison with other procedures. Applied and Environmental Microbiology, 46, 491–498.

Dagg, M.J., Walser, W.E., Jr. 1987. Ingestion, gut passage, and egestion by the copepod *Neocalanus plumchrus* in the laboratory and in the subarctic Pacific Ocean. Limnology and Oceanography, 32, 178–188.

Evans, G.T., Parslow, J.S. 1982. Metal contamination and its effect on primary production measurements. Limnology and Oceanography, 27, 544–551.

Fitzwater, S.E., Knauer, G.A., Martin, J.H. 1982. Metal contamination and its effect on primary production measurements. Limnology and Oceanography, 27, 544–551.

Frost, B.W. 1987. Grazing control of phytoplankton stock in the open subarctic Pacific Ocean: a model assessing the role of mesozooplankton, particularly the large calanoid copepods, *Neocalanus* spp. Marine Ecology Progress Series, 39, 49–68.

Frost, B.W., Landry, M.R., Hassett, R.P. 1983. Feeding behaviour of large calanoid copepods *Neocalanus cristatus* and *N. plumchrus* from the subarctic Pacific Ocean. Deep-Sea Research, 30, 1–13.

Grice, G.D., Reeve, M.R., ed. 1982. Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 430 pp.

Heinrich, A.K. 1957. [The breeding and development of the dominant copepods in the Bering Sea.] Trudy Vsesoyznzogo Gidrobiologicheskogo Obshchestra, 8, 142–162. [In Russian.]

______ 1962. The life histories of plankton animals and seasonal cycles of plankton communities in the oceans. Journal du Conseil International pour l'Exploration de la Mer, 27, 15–24.

Landry, M.R., Haas, L.W., Fagerness, V.L. 1984. Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. Marine Ecology Progress Series, 16, 127–133.

Lorenzen, C.J. 1966. A method for the continuous measurement of in vivo chlorophyll concentration. Deep-Sea Research, 13, 223–227.

Martin, J.H., Fitzwater, S.E. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature, 331, 341–343.

McAllister, C.D., Parsons, T.R., Strickland, J.D.H. 1960. Primary productivity and fertility at station "P" in the northeast Pacific Ocean. Journal du Conseil International pour l'Exploration de la Mer, 25, 240–259.

Miller, C.B., Frost, B.W., Batchelder, H.P., Clemons, M.J., Conway, R. 1984. Life histories of large, grazing copepods in the subarctic ocean gyre: *Neocalanus plumchrus*, *Neocalanus cristatus*, and *Eucalanus bungii* in the northeast Pacific. Progress in Oceanography, 13, 201–243.

Parsons, T.R., LeBrasseur, R.J. 1968. A discussion of some critical indices of primary and secondary production for large scale ocean surveys. Californian Marine Research Communications, CalCOFI 12, 54–63.

Parsons, T.R., Giovando, L.F., LeBrasseur, R.J. 1966. The advent of the spring bloom in the eastern subarctic Pacific Ocean. Journal of the Fisheries Research Board of Canada, 23, 539–546.

Porter, K.G., Feig, Y.S. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography, 25, 943–948.

Welschmeyer, N.A., Lorenzen, C.J. 1985. Chlorophyll budgets: zooplankton grazing and phytoplankton growth in a temperate fjord and the Central Pacific gyres. Limnology and Oceanography, 30, 1–21.

Fate of Petroleum Hydrocarbons in Marine Ecosystem Enclosures and Relevance to Marine Oil Spills

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In this review, stimulation of the growth of native microbial populations through the introduction of crude oils, refined oils, and individual hydrocarbons into marine enclosures is examined. Also, the involvement of microbes in the degradation of alkanes and aromatic hydrocarbons is assessed. Finally, a case is presented for a possible pivotal role for microbial flocs in biodegradation of petroleum hydrocarbons at sea.

In the late 1960s and early 1970s, the continuing increase in oil-tanker traffic, reports of floating tar balls far out at sea, evidence of chronic oil releases in marine operations, the potential for catastrophic oil spills, and a lack of knowledge concerning the fragility of the marine biosphere pressed the scientific community to gather evidence on the fate and effects of petroleum hydrocarbons in marine ecosystems. Pursuit of this evidence continues to this day. The marine enclosure now being used in its many forms by researchers around the world has afforded an opportunity to study the fate and effects of petroleum and other pollutants on marine communities under controlled conditions.

This paper focuses on three aspects of the fate and effects of petroleum hydrocarbons in marine enclosures: stimulation of microbial growth, biodegradation of dispersants and petroleum hydrocarbons, and the role of bioflocs in oil biodegradation and sedimentation. In addition, this paper examines the consequences of using a chemical dispersant, specifically Corexit 9527, to facilitate the transport of oil into the water column.

Stimulation of microbial growth

In one of the first oil pollution experiments (Hodson et al. 1977) carried out in a Controlled Ecosystem Pollution Experiment (CEPEX) enclosure in August 1974, No. 2 fuel oil at an initial concentration of 0.01 $mg \cdot L^{-1}$ caused neither an increase nor a decrease in the microbial population over a 30-d experiment. At that concentration, the fuel oil did not stimulate or inhibit bacterial growth. In contrast, the addition of Cu^{+2} at 0.01 and 0.05 $mg{\cdot}L^{-1}$ resulted in a dramatic increase in bacterial numbers. The preferred explanation for the increase was that population growth was stimulated by the rapid release of heterotrophic substances resulting from increased algal excretion and mortality (Vaccaro et al. 1977).

Thus, it appeared that No. 2 fuel oil had little effect on populations growth, either directly by affecting bacteria or indirectly through algal toxicity, at a concentration of 0.01 $mg{\cdot}L^{-1}$. That concentration of fuel oil appeared not to increase the potential of the indigenous microbial population to degrade hydrocarbons either, based on the ability to oxidize in vitro [$n^{14}C$]fluorene and [$n^{14}C$]hexadecane. Furthermore, with D-glucose assimilation as an index, the data obtained using several different oils and water samples drawn from control and oil enclosures after 30 d indicated that a concentration of oil greater than about 0.3 $mg{\cdot}L^{-1}$ would inhibit the activity of natural marine bacterial populations.

In apparent contrast to the results of the early CEPEX experiment, Hagstrom (1977) reported greater than 100-fold increases in bacterial numbers over 10–15 days in a 4.2-m^3 enclosure equipped with a flow-through system delivering Baltic seawater (7‰ salinity) under a slick of light fuel oil. The concentration of oil under the slick varied from about 1–9 $mg{\cdot}L^{-1}$ depending on weather conditions. Although a number of differences, such as salinity, volume, water source, and method of conducting the experiment, distinguished this experiment from the CEPEX experiment, temperatures were at least comparable and the oils were similar in that No. 2 fuel oil is a light fuel oil. The major difference appears to have been the concentration of oil in the water, i.e., 0.01 $mg{\cdot}L^{-1}$ initially for the CEPEX experiment and 1–9 $mg{\cdot}L^{-1}$ for the Baltic seawater study.

Another enclosure study showing microbial growth in response to oil was conducted in France. In this particular study (Marty et al. 1979), topped Saudi Arabian crude oil was mixed into 15 m^3 of seawater in 20-m^2 circular basins with the aid of chemical dispersants. The dispersants were Corexit 9527, Hydrogamosol LT, and OSR LT 126. The concentrations of the oil over the course of the experiment were not reported. Given complete dispersion, the initial concentration could have been over 600 $mg{\cdot}L^{-1}$. The concentration of oil would have fallen rapidly, however, as the dispersant could not have maintained such a high concentration for very long. By the next day, a fairly long-lasting dispersion in water (in the 1–10 $mg{\cdot}L^{-1}$ range) would have been likely (Cretney et al. 1981; MacNeill et al. 1985).

Bacterial growth during the first 15 d was observed in all treatment tanks, whereas none was seen in the seawater control. There was a varying lag period of 1–3 d with respect to bacterial growth for the treatments involving oil and dispersant mixtures. In the case of the Corexit 9527-generated oil dispersions, after a 1-d lag in one experiment and a 3-d lag in another, increases were about 100-fold and 10-fold, respectively, after 15 d. In the case of treatments with oil or dispersants only, there was no lag in bacterial growth, so the most rapid growth was obtained during the first 24 h. In the case of Corexit 9527, a 40–120-fold increase in population was observed during this initial period. The causes of the bacterial population increase may have been multifold, arising from utilization of the carbon sources presented by the dispersant or oil or both, utilization of algal products released under dispersant or oil stress, or elimination of bacterial predators. The lag phase in the case of the mixed oil and dispersant treatments may have reflected toxicity to microbes of high initial concentrations of oil in the seawater. The absence of a lag in the growth of bacteria in the tank treated only with oil may have resulted from inefficient transfer of the topped oil to the water column and the short period of exposure to the toxic substance.

In an experiment carried out in 1977 in Patricia Bay, Saanich Inlet, British Columbia, using 66-m^3 enclosures of the CEPEX design (Cretney et al. 1981), immediate logarithmic bacterial growth was observed 2 m beneath a slick of Prudhoe Bay crude oil that was premixed with Corexit 9527 (20:1). The same phenomenon also occurred when a weathered slick in a second enclosure was sprayed with Corexit 9527. The increase in bacteria was about 100-fold whereas the presence of an oil slick itself resulted in less than a fivefold increase. The chemically dispersed oil was carried into the water column by turbulent diffusion, which generated approximately Gaussian concentration profiles. For most of the experimental period, the concentrations were in the range of 1–20 mg·L^{-1}. As in the other studies cited, the reason for the bacterial growth was not elucidated. Before the oil was placed in the enclosures, however, they were allowed to stand for 5 d. During this time, a phytoplankton bloom occurred and crashed, after which the settled material was removed at the bottom. The seawater in the enclosure was then thought to be nutrient depleted without a significant phytoplankton population. Although bacterial growth following dispersion of the oil could have been stimulated by the presence of a carbon source in the form of oil hydrocarbons or the dispersant, elimination of bacterial grazers was a more likely reason.

A much more complete study of the fate and effects of dispersion of Prudhoe Bay crude oil by Corexit 9527 in Patricia Bay using 66-m^3 enclosures was carried out in July–August 1983 (Parsons et al. 1984; Wong et al. 1984; Lee et al. 1985; Harrison et al. 1986). In this experiment, the oil–dispersant mix was injected at a depth of between 2 and 4 m to generate an initial concentrations of oil at 20 mg·L^{-1} and of dispersant at 2 mg·L^{-1}. In another enclosure, a similar injection was made with dispersant only. A third enclosure was used as a control. Inorganic nutrients were added to all enclosures to stimulate phytoplankton growth in a departure from the 1977 study. Primary production was stimulated immediately in the control and Corexit-only enclosures, but was much delayed in the oil–dispersant enclosure. The oil–dispersant enclosure showed an almost immediate increase in bacterial standing stock and heterotrophic bacterial production, each reaching a maximum after about 5 d. The other enclosures showed similar increases in heterotrophic potential, but without the increases in standing stock. This apparent discrepancy was most likely caused by grazing pressure of bacteriovores that were controlling the population in the control and Corexit enclosures, but were eliminated or incapacitated in the Corexit–oil enclosure (Lee et al. 1985). Phasing of bacterial growth and the reduction of zooflagellates in each of the three enclosures was consistent with this grazing hypothesis. In general, the control enclosure and the enclosure treated only with Corexit behaved in a similar manner with respect to plankton dynamics. The small quantitative differences observed were within the range of natural variability.

In March and April 1979, at Rosfjord in southern Norway, an enclosure study similar to the 1977 Patricia Bay study was carried out using Ekofisk crude oil (Laake et al. 1984). No chemical dispersant was used, but natural mixing action generated oil concentrations in the 1–20 mg·L^{-1} range. Bacterial growth was observed and the population reached a maximum at 6 m after 4 d. The approximately 10-fold increase in bacterial population gradually diminished in succeeding days to a minimum between days 9 and 10 before once again increasing. The bacterial maximum followed the initial oiling and subsequent crash of the resident phytoplankton population. The minimum after 9 d was attributed to a low concentration of dissolved nitrogen and phosphorus. An increase in bacterial numbers after day 9 coincided with the mixing in of nutrients from below 10 m on days 10 and 11. No information on zooplankton dynamics was presented, so that the probable influence of grazing pressure cannot be determined.

In general, marine enclosure work indicates that stimulation of bacterial growth will occur with oil concentrations in the low-milligrams per litre range. Concentrations in the low-micrograms per litre and high-milligrams per litre ranges seem to elicit "no response" and "inhibition of growth" respectively. If nutrient supply from affected phytoplankton and elimination of bacteriovores are important general consequences of oil that is naturally, mechanically, or chemically dispersed, as indicated by the enclosure studies, bacterial growth in the wake of oil slicks would seem to be a reasonable consequence of oil spills at sea. In this regard, it is worth noting the results of a 100-t experimental oil spill that was conducted in 1982 at Halten Bank, off Norway (Lange 1985). The oil appeared to inhibit bacterial growth under the thickest part of the slick. The bacterial concentration under the tail of the slick, however, was about 10 times the concentration found in unoiled seawater at the same depth. Lange speculated that the exposure time of 1.5–3 d may have been sufficient to result in the observed bacterial increase.

Biodegradation of oil dispersants and oils

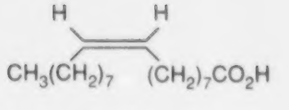
Oil dispersant biodegradation

The formulation of oil-spill dispersants tends to be proprietary information that is not readily available from manufacturers. The basic formulation of Corexit 9527 (Table 1, Fig.1), manufactured by Exxon (Canevari and Cranford 1974), is probably typical of most dispersants, however, and is available to researchers (Wells et al. 1985).

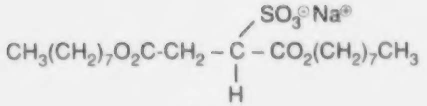
Oleic acid (I) is a natural fatty acid with a central olefinic bond and 18 carbon atoms. It is the most common fatty acid constituent of olive oil, peanut oil, beef tallow, and whale blubber (Fieser and Fieser 1961). Sorbitan monooleate (II), also known as Span 80, is used in pharmaceutical formulations. The sorbitan monooleate ethylene oxide adduct (20 mol) is also known as Tween 80. This compound is used as an emulsifier and dispersant in pharmaceutical products that are meant to be taken internally. The fatty acid component of Tween 80 and Span 80 is oleic acid. Sodium dioctylsulfosuccinate or Aerosol OT is a typical wetting agent (Fieser and Fieser 1961). The glycol ether may be a cellusolve, such as Cellusolve (2-ethoxyethanol) (IV, R=CH_3CH_2) or Butylcellusolve (2-butoxyethanol) (IV, R=$CH_3(CH_2)_3$), which are common industrial solvents.

Table 1. Composition of Corexit 9527.

Compound	Composition (%)
Oleic acid (I) (and/or sorbitan monooleate (II))	16
Sorbitan monooleate ethylene oxide adduct (20 mol)	32
Sodium dioctylsulfosuccinate (III)	35
Glycol ether	17



I

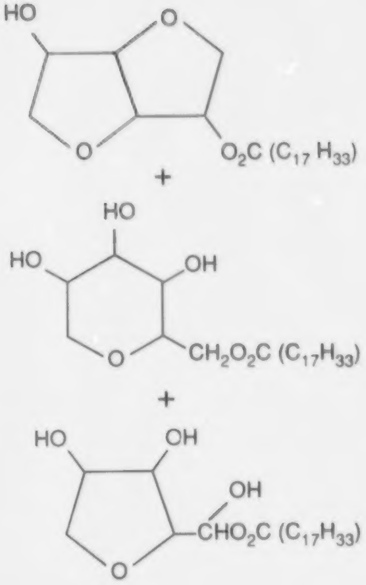


$CH_3(CH_2)_7O_2C\text{-}CH_2-\underset{H}{\overset{SO_3^{\ominus}Na^{\oplus}}{C}}-CO_2(CH_2)_7CH_3$

III

$HO-CH_2\,CH_2-OR$

IV



II

Fig. 1. Constituent components of Corexit 9527.

Regarding biodegradation of compounds such as oleic acid, Tween 80, and Span 80, an interesting paper by Una and Garcia (1983) is available. They studied biodegradation of nonionic dispersants in seawater in the laboratory using microorganisms obtained from an oil-spill site. They proposed a kinetic model of enzyme action based on their results that involved hydrolysis of the ester, followed by cleavage of the double bond in oleic acid to give nonanoic acid ($CH_3(CH_2)_7CO_2H$) and azaleic acid ($HO_2C(CH_2)_7CO_2H$). These two acids are further oxidized by β-oxidation in a cascade to acetate. Based on the kinetics they observed, Una and Garcia proposed a model with two remarkable attributes:

- The hydrolysis of the ester is sterically impeded by the ethylene oxide residues or hindered by their promoting the formation of micelles.
- Once cleavage has occurred, however, the ethylene oxide-containing hydrolysis product promotes oxidative cleavage of the oleic acid.

Formulation of Corexit 9527 by accident or design thus seems ideally suited to biodegradation of its principal components.

Using 38-L aquaria in a laboratory study, Traxler and Bhattacharya (1978) obtained evidence of the biodegradation of Corexit 9527 in the presence and absence of crude oils by unaltered microbial populations of seawater from Narragansett Bay, RI, USA. Oxygen depletion by microorganisms occurred in the presence of Corexit 9527 as the only carbon source. In a series of experiments using n-[1-^{14}C]hexadecane in Kuwait crude oil, these workers observed a 20-h lag in test systems containing dispersant before the labeled n-hexadecane was oxidized to carbon dioxide. This lag corresponded to the time previously found necessary for biodegradation of the dispersant. They concluded that Corexit 9527 was biodegradable and was not toxic to hydrocarbon degraders.

Another laboratory study (Foght and Westlake 1982) showing temporary retardation of oil degradation by Corexit 9527 also established that the length of the lag period depended on the amount of Corexit 9527 present. The length of the lag also depended on the nutrient state. In the case of no nutrient supplementation, in flasks with 10% Corexit 9527, there was essentially no degradation of n-alkanes after 12 weeks; whereas in flasks with 0 or 1% dispersant, degradation of these components was complete.

Hydrocarbon biodegradation

In this section, those enclosure studies in which a direct measure of hydrocarbon biodegradation in the water column has been made are specifically mentioned. Such direct measures include determination of ratios of n-alkane to isoalkane, $^{14}CO_2$ activity, and ^{14}C activity incorporated into biota other than in the form of the originally labeled hydrocarbon.

Biodegradation of aromatic hydrocarbons

In a CEPEX enclosure experiment carried out during the summer of 1976, Lee et al. (1978) added radioactive [^3H]benzo[a]pyrene (Fig.2: V) to determine its fate in the controlled ecosystem. About 40% of the labeled benzo[a]pyrene was found in the bottom sediments. Evaporation was discounted as a possible reason for the loss of the remainder. Incomplete recovery from the sediments and some loss to the enclosure walls probably occurred, but these losses were not considered to be important enough to account for the missing radioactivity. During the experiment, however, 70–90% of the radioactivity in the water was removed by filtration using glassfibre filters. Of the nonfilterable radioactivity, a portion from 0 to 67% could be attributed to hydroxylated derivatives and quinones. Based on measurements of biodegradation potential using water samples from the enclosures, biodegradation was considered to be unimportant as a removal process for benzo[a]pyrene. The researchers concluded that photochemical oxidation, rather than biochemical oxidation, probably accounted for the removal of up to 50% of the labeled benzo[a]pyrene.

In a 1978 MERL experiment (Hinga et al. 1980), the biogeochemical fate of ^{14}C-labeled benz[a]anthracene (VI) was determined. The radiotracer label was followed for 230 d. The production of $^{14}CO_2$, and concentrations of labeled benzo[a]anthracene and its oxidative products were determined. Hinga et al. (1980) proposed that a combination of microbial and photochemical processes gave rise to the labeled products found in the water and sediments. They also suggested that animal metabolism might play a degradative role in sediments. These authors did not attempt to estimate the relative importance of photooxidation and biooxidation in the degradation of the hydrocarbon. In this regard, it should be noted that the benz[a]anthracenes were transported to the sediments within the first few days of the start of the experiment and that most of the mineralization of the compound occurred there. The authors calculated that the half-life of benzo[a]anthracene fell within the range of 1.2–3 years. In a later experiment using 7,12-dimethylbenz[a]anthracene, Hinga et al. (1986) found that photodegradation probably accounted for the initial

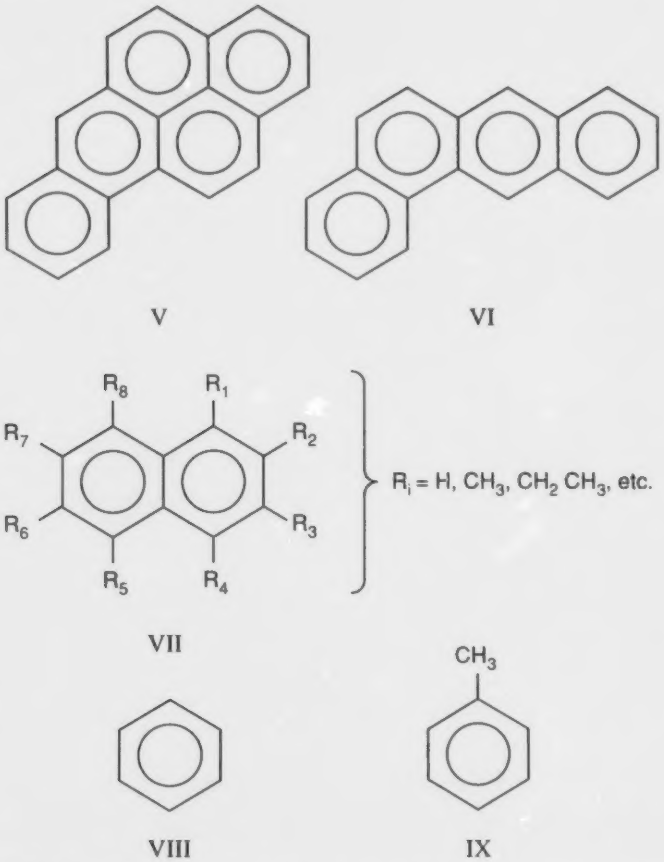

V VI VII VIII IX

Fig. 2. Aromatic hydrocarbons that have been studied in mesocosm experiments.

transformation of that compound in the water column, with biodegradation probably being responsible for mineralization.

In an earlier series of three experiments beginning in December 1976 and running with intervals in between through to July 1978 (Gearing et al. 1979), evidence of biodegradation of naphthalenes (VII) was obtained. In the latter two experiments, semiweekly oil additions were carried out for several months. The degradation potential for the naphthalenes was found to be enhanced by the semiweekly additions of oil. This finding was backed up by depletion of naphthalenes in the water column, attributable to biodegradation, which correlated nicely with the biodegradation potentials determined. No rates were calculated, but significant changes, due to biodegradation, were observed in 3–4 d.

From March 1982 to September 1983, a series of 10 radiotracer experiments were conducted using the MERL system (Wakeham et al. 1986a). All but three of the experiments involved aromatic hydrocarbons or halogenated aromatic hydrocarbons. Removal rates were determined for the compounds. First-order rate constants for biodegradation of benzene (VIII), toluene (IX), and naphthalene (VII, Ri = H for i = 1 ... 8) were determined. The rate constant's values depended on the time of the year for which they were determined, the method of calculation, and also on the time interval from the start of the experiment over which they were calculated. The maximum rates, however, were 1.67, 1.07, and 1.17 d^{-1} for benzene, toluene, and naphthalene respectively.

Biodegradation of n-alkanes

During the series of experiments (Gearing et al. 1979) described above in which evidence of naphthalene biodegradation was obtained, biodegradation of n-alkanes was also evident from the changing ratios of n-alkanes to isoprenoids in water samples collected from the enclosures. Adaptation of the microbial population to oil was also indicated. For example, in a comparison of the hydrocarbon distributions determined for water samples obtained 90 h after the 3rd and 33rd oil additions, the n-alkane to isoprenoid ratios were much reduced after the 33rd addition, but only slightly reduced after the 3rd addition compared with those ratios observed when No. 2 fuel oil was added to the ecosystem. The authors did not attempt to determine rate constants or half lives for the n-alkanes. Given that there was no degradation of the isoprenoids and that simple first-order kinetics apply, a rough estimate of 0.17 d^{-1} for the rate constant can be made using the n-heptadecane to pristane ratio (Table 2).

In a series of experiments designed to examine transportation of No. 2 fuel oil between the water column, surface microlayer, and atmosphere in a MERL enclosure, the relative extents of biodegradation occurring in the water column and surface microlayer were compared (Gearing and Gearing 1982). No evidence of heightened microbial oxidation of oil in the surface microlayer was obtained. A temperature effect was observed, however, so that enhanced degradation was observed in the warm waters of the July 1978 and September 1979 experiments compared with the cold water of the March 1978 experiments.

Although the data are perhaps unsuitable for rate-constant determinations, a crude calculation, to provide a basis for comparison, gives a first-order rate constant of 0.05 d^{-1} for the March (0–2°C) water and constants of 0.32 and 0.19 d^{-1} for the warmer waters of July (20–21°C) and September (17–19°C) respectively. These estimates are calculated from the ratios of n-hexadecane to norpristane and are subject to error arising through evaporation and dissolution during the course of the experiment. Norpristane has a higher vapour pressure and greater solubility than n-hexadecane at ambient temperature, so these processes tend to increase the ratio of n-hexadecane to norpristane with time as biodegradation reduces it. The same problem exists for the other n-alkane to isoprenoid ratios used; so with all other factors being equal, the rate constants calculated would tend to underestimate the actual values.

In the March 1982 to September 1983 series of radiotracer experiments on biodegradation of aromatic hydrocarbons using MERL enclosures, biodegradation of n-decane and n-octadecane was also studied (Wakeham et al. 1986b). Depending on whether the rate constant was determined from $^{14}CO_2$ production in situ or loss of radioactivity in situ that was not accounted for by evaporation or sedimentation or loss mechanisms other than mineralization, rate constant values were 1.21 or 1.26 d^{-1} for n-decane and 0.66 or 0.34 d^{-1} for n-octadecane.

Table 2. Biodegradation of n-alkanes in marine enclosures.

Site	Reference	Date	Water temperature (°C)	Hydrocarbon source	Means of dispersion	Biodegradation rate (per day)
MERL[a]	Gearing et al. (1979)	May/June 1978	8–10	No. 2 fuel oil	Mechanical	0.17^{b}
MERL	Gearing and Gearing (1982)	Mar. 1978	0–2	No. 2 fuel oil	Mechanical	0.05^{c}
MERL	Gearing and Gearing (1982)	July 1978	20–21	No. 2 fuel oil	Mechanical	0.32^{c}
MERL	Gearing and Gearing (1982)	Sep. 1978	17–19	No. 2 fuel oil	Mechanical	0.19^{c}
MERL	Wakeham et al. (1986b)	May 1983	11–14	n-decane	Mechanical	1.21^{d} 1.26^{e}
MERL	Wakeham et al. (1986b)	Sep./Oct. 1982	13–18	n-octadecane	Mechanical	0.66^{d} 0.34^{e}
Patricia Bay	Cretney et al. (1981)	Sep. 1977	12–13	Prudhoe Bay crude oil	Corexit 9527	0.27^{f}
Patricia Bay	Wong et al. (1984)	July/Aug. 1983	12–15	Prudhoe Bay crude oil	Corexit 9527	0.34^{d}
Xiamen Bay	Li W., et al. (this volume) Wu et al. (this volume)	June 1986	20–26	Shengli crude oil	Corexit 9527	0.41^{g} 0.46^{h} 0.37^{e}

[a] Marine Ecosystems Research Laboratory, University of Rhode Island, USA.

[b] Calculated from n-heptadecane to pristane ratio.

[c] Calculated from n-hexadecane to norpristane ratio.

[d] Calculated from $^{14}CO_2$ released from labeled n-alkane.

[e] Calculated from radioactivity remaining in system other than $^{14}CO_2$.

[f] Calculated from n-octadecane to phytane ratios.

[g] Calculated from n-hexadecane to norpristane, n-heptadecane to pristane, and n-octadecane to phytane ratios.

[h] Calculated from n-heptadecane to pristane and n-octadecane to phytane ratios.

In the 1977 experiment conducted in Patricia Bay in which dispersion of Prudhoe Bay crude oil with Corexit 9527 was studied, the ratio of n-octadecane to phytane was monitored as the means of detecting microbial degradation (Cretney et al. 1981). This ratio remained essentially constant for a period of at least 4 d. Between day 4 and day 8, the ratio decreased to almost one-third of its initial value and continued to decrease to the end of the experiment. The total bacterial population apparently increased by more than an order of magnitude up to day 4 and by more than another order of magnitude by day 8 before the population entered senescence. Thus, most of the biodegradation appeared to occur during the logarithmic growth phase of the bacteria. Of course, total bacterial dynamics do not necessarily reflect those of the oil-degrading microbial population. Indeed, an extended linear growth phase is often observed for microorganisms growing on low-solubility hydrocarbons such as n-hexadecane (Mallee and Blanch 1977).

By ignoring bacterial growth and assuming simple zero- or first-order kinetics for biodegradation after the lag phase, a rate constant of 0.13 d^{-1} (relative) or 0.27 d^{-1}, respectively, can be calculated for the degradation of n-octadecane and by inference the other n-alkanes, which in the study all appeared to be used at about the same rate.

The lag phase may have been a result of earlier bacterial use of Corexit 9527, in keeping with the studies of Traxler and Bhattacharya (1978) and Foght and Westlake (1982). The lag phase may also have been a reflection of the time required for the growth of an n-alkane degrading population or the formation of bioflocs or both.

In the 1983 experiments in Patricia Bay using Prudhoe Bay crude oil and Corexit 9527 as a dispersant, n-[1-^{14}C]hexadecane was added to the oil to provide a measure of biodegradation other than the ratio of n-alkane to isoprenoid. In this experiment, there was a 5- to 6-d lag before biodegradation was indicated by $^{14}CO_2$ evolution and a decrease in the ratio of n-alkane to isoprenoid. In contrast to the previous experiment, initiation of biodegradation coincided with the maximum in bacterial standing stock and production. Furthermore, biodegradation proceeded as these two measures decreased, which was also in contrast to the previous experiment. As noted above, however, it is the standing stock and activity of the oleoclastic bacteria, not the total bacteria, that one would expect to be closely coupled with biodegradation. Production of $^{14}CO_2$ between day 6 and day 8, after dispersion of the oil, can be used to calculate the first-order rate constant for biodegradation. This estimate is 0.34 d^{-1}. The average rate constant for biodegradation calculated from the n-hexadecane to norpristane, n-heptadecane to pristane, and n-octadecane to phytane ratios is 0.41 d^{-1}.

In an experiment performed in China in May–June 1986 using 14-m^3 marine enclosures in the eastern part of Xiamen Bay, n-alkane biodegradation was again observed (Li W., et al., this volume; Wu S., et al., this volume). In this experiment, Shengli crude oil was dispersed with Corexit 9527 (Zhuang et al., this volume). In contrast to the results of the two experiments conducted in Patricia Bay, biodegradation commenced with almost no lag period. The maximum rate was observed after only 1 d based on ratios of n-alkane to isoprenoid. An average rate constant of 0.46 d^{-1} was determined for the particulate (filtered) oil from n-heptadecane to pristane and n-octadecane to phytane ratios.

In many enclosure experiments in which evidence of n-alkane biodegradation has been sought, it has been found. Remarkably, for whatever reasons, the rate constants have fallen in quite a narrow range. The consistency of the results from enclosure studies establishes that the microbial population will respond positively to oil in the water column under a variety of conditions encompassed by a variety of enclosure designs, geographical locations, oil compositions, and means of dispersion. Further work in marine enclosures should address the mechanistic aspects of degradation to increase the predictive capability of enclosure experiments with respect to bacterial response to actual oil spills.

Role of bacterial flocs in oil biodegradation

An important consideration pertaining to the mechanism of biodegradation of petroleum oil hydrocarbons at sea is the relevance of bacterial floc formation. For the purpose of this discussion, a bacterial floc is an agglomeration of bacteria, oil as discrete particles, particulate organic material (POM), and sometimes air bubbles. Several advantages may accrue to oil-degrading microorganisms through floc formation:

- First, the hydrocarbon substrate is kept close to the bacterial cells under conditions in which global dilution, for example by diffusive mixing under an oil slick, would tend to separate unattached bacteria and substrate.

- Second, the floc surface may concentrate inorganic nutrients essential to the microorganisms (Velankar et al. 1975; Shanks and Trent 1979; Hebel et al. 1986).

- Third, inclusion of phytoplankton in a bacterial floc would facilitate the interchange of chemicals (Escher and Characklis 1982). For example, a close coupling would occur between O_2 released by phytoplankton and required for bacterial respiration and CO_2 released by bacteria and required for phytoplankton photosynthesis.

- Fourth, hydrocarbon-degrading microorganisms, including bacteria, are generally capable of producing nonspecific oil-emulsifying agents and specific solubilizing agents that selectively carry particular hydrocarbons (Velankar et al. 1975; Reddy et al. 1982, 1983; Swaranjit et al. 1984). Floc formation would retard the loss of these agents to their environment (Atkinson and Rahman 1979) and create a microenvironment for hydrocarbon assimilation (Kulkarni and Barnett 1979). The floc represents a superentity in which hydrocarbon processing is effectively internalized.

- Fifth, aggregation of bacteria increases their settling velocity and their potential for absorbing nutrients (Csanady 1986; Logan and Hunt 1987). In this regard, collection of finely dispersed oil droplets would also be enhanced. Advection to deeper water may be controlled by complete or partial disaggregation, i.e., generation of smaller particles or more porous, less dense, particles. Some large marine snow particles (4–5 mm in diameter) have been observed to sink very slowly ($1\ m \cdot d^{-1}$) because of their low density (Asper 1987).

- Sixth, in general, the ability of microorganisms to adhere to surfaces or to detach from them appears to be a survival response elicited by starvation

(Loosdrecht et al. 1987). In the case of oleoclastic bacteria presented with an insoluble hydrocarbon substrate, floc formation would seem to be a natural manifestation of this survival strategy, especially when the hydrocarbon is dispersed in a size range with an upper limit not many-fold larger than the bacteria. At the same time, once the hydrocarbon has been completely respired or assimilated, detachment would be an appropriate survival response, freeing the bacteria to find and attach to other sources of carbon. Disintegration of flocs into free cells after complete use of the hydrocarbon substrate and conversion of extracellular material into intracellular products has been observed during microbial growth in bioreactor experiments (Mallee and Blanch 1977).

- Seventh, the floc may protect the cells from predation and competition. As in the case of solubilizing and emulsifying agents, loss of protective toxins to the environment would be retarded. Loss of autotoxic wastes, however, may also be retarded. In addition, the framework of the floc may present an obstruction to some predators and toxins of competitors. Although floc size may make the bacteria susceptible to predation by higher trophic level consumers (Biddanda 1985, 1986), spreading feeding pressure among many consumers may enhance overall survivability. In this regard, cyclic aggregation and disaggregation could permit a bacterial population to stay a step ahead of consumer populations that prefer prey of specific limited size ranges.

Floc formation, however, is not without probable disadvantages for oil-degrading microorganisms:

- First, mass transport of hydrocarbons into and within flocs may be diffusion limited (Atkinson and Rahman 1979; Benefield and Molz 1983; Csanady, 1986). Because molecular diffusion varies inversely with molecular weight (Glasstone and Lewis 1960), hydrocarbons carried by solubilizing agents, which can have molecular weights in the 10^5–10^6 Dalton range, or in colloidal-sized or larger micelles (Zosim et al. 1982; Cameotra et al. 1983; Reddy et al. 1983) may be severely diffusion-limited inside flocs. This limitation, however, assumes that the floc interior is stagnant and not influenced by the exterior flow field. There is evidence, however, that microbial aggregates may be sufficiently porous to allow fluid flow through them (Ho et al. 1984; Logan and Hunt 1987). Also, deformation of the flocs and disaggregation–reaggregation processes may serve to alter concentration gradients within them (Atkinson and Rahman 1979).

- Second, even in the presence of hydrocarbon substrate within a floc, a portion of nutrients and oxygen (which is not supplied by any associated organisms) must be obtained from the surrounding medium. If the floc is a three-dimensional ovoid or similar shape, the larger the floc, the more serious the problem of diffusive transport to its centre (Atkinson and Rahman 1979; Benefield and Molz 1983). Indeed, oxygen depletion has been observed directly in marine snow particles of 1-mm diameter or greater (Alldredge and Cohen 1987). Microbial flocs, and marine snow particles for that matter, may not have a well-defined, three-dimensional structure, however. They may have an effective or fractal dimension between 0 and 3 (Mandelbrot 1983). Once the bacteria produced an exocellular adhesive polymer, flocs would form through a random growth process of cluster-by-cluster aggregation to give scale-invariant structures with a fractal dimension (assuming no annealing) between 1.6 and 2.2 (Witten and Cates 1986). The floc structure

> would be sponge-like. The highly porous flocs would be permeable to flow from the surrounding medium. External fluid would flow through them while they settled under gravity or were subjected to turbulent shear.

Evidence for possible involvement of biological floc formation during oil biodegradation in enclosures rests on experiments carried out in marine enclosures in Patricia Bay, Vancouver Island, BC, Canada. In controlled ecosystem enclosure experiments in which Prudhoe Bay crude oil augmented with several aromatic hydrocarbons was used, Lee et al. (1978) observed that naphthalene and its mono- and dimethyl derivatives were transported rapidly to the sediments during the first days of the experiment, whereas higher molecular weight compounds settled more slowly, arriving during the final days of the experiment. They proposed that the naphthalenes were actively taken up by the phytoplankton and settled out with them during the first days of the experiment. On the basis of autoradiographic studies with labeled aromatic hydrocarbons, they also concluded that higher molecular-weight hydrocarbons were associated with detrital material that was "composed of dead phytoplankton cells and bacteria," and presumably oil particles. They also suggested that these aggregates settled more slowly than the phytoplankton cells, accounting for the slower descent to the sediments of higher molecular-weight aromatic compounds.

In a subsequent experiment in Patricia Bay using Prudhoe Bay crude oil dispersed into a marine enclosure with Corexit 9527 (Cretney et al. 1981), bacterial floc formation concomitant with biodegradation of the n-alkane components of the oil was observed. Toward the end of the experiment, flocs were present that were 1 cm or more in diameter. The concentration of these flocs at the end of the experiment was so high that divers cutting away samples of the enclosure walls for further analysis commented that water coming out of the enclosures "looked like a sleet storm." It should be noted that, in this experiment, sufficient time was provided for the phytoplankton to bloom, settle out, and be removed from the bottom before the addition of oil. No nutrients were added to stimulate new phytoplankton growth. Of the crude oil that was added, most remained at the surface or in suspension. Very little sedimented out or adhered to the enclosure walls.

In an experiment conducted in 1983 using Prudhoe Bay crude oil and Corexit 9527 (Parsons et al. 1984; Wong et al. 1984; Lee et al. 1985; Harrison et al. 1986), bacterial flocs were also observed. In this experiment, nutrients were added to stimulate phytoplankton growth. Although rapid sedimentation of oil was observed, in contrast to the previous experiment, sedimentation coincided with the settling out of pennate and centric diatoms. Phase-contrast and epifluorescent microscopy of sedimenting material (Wong et al. 1984; Lee et al. 1985) showed that discrete oil particles in the ≤0.2–3 μm range were held in close association by what seemed to be an immobilizing organic matrix. These aggregates of oil particles and bacteria were also associated with centric and pennate diatoms whose siliceous exterior presumably provided ballast to permit more rapid sedimentation than would be possible for a bacteria-only aggregate. The nature of the interaction between the flocs and diatoms is unclear, but particularly in the case of the centric diatoms, where the flocs seemed to be impaled on their silica spines, the association may be purely mechanical. Thus, sedimentation of the oil-containing flocs may be attributable to a mechanical sweeping of the water column by sedimenting diatoms and to chemical adhesion through bacterial exudates (Iman et al. 1984; Lewin 1984; Paul and Jeffrey 1985; Loosdrecht et al. 1987). It should be noted, however, that diatoms may aggregate through mucous secretions and undergo physiological changes to increase sinking rates (Smetacek 1985).

A noteworthy characteristic of the 1983 experiment was a shift in the maximum of ^{14}C activity added in the crude oil as radiolabeled n-hexadecane from the 0.45–3 to 3–8 μm size ranges (Wong et al. 1984; Lee et al. 1985). This shift coincided with the peaks in bacterial activity, bacterial standing stock, and incorporation of activity in polysaccharide, nucleic acid, protein, and low molecular-weight components of the biological materials. Although the involvement of bacteria in the initial stages of the association of oil particles (generally in a size range of ≤0.2–3 μm) with POM is open to question, the bacteria seem likely to have been responsible for the formation of larger aggregates from smaller ones that originally contained the radiolabel. Following the rapid release of $^{14}CO_2$ between day 7 and day 9, the peak in ^{14}C activity shifted back to the size range of smaller POM. This occurred even though the trend to larger particles continued (as shown by Coulter Counter results) indicating, perhaps, that respiration of $^{14}CO_2$ was more efficient in the larger particles. Because alternative explanations are possible, however, this explanation must await confirmation in other studies.

The mesocosm experiments conducted in Patricia Bay have indicated that, at least at this site, bacteria play an active role in the formation of an organic matrix much larger than the nominal size of bacteria or oil particles that have been generated by a chemical dispersant. The evidence has not established that the hydrocarbons in these oil particles are biodegraded by the attached or encapsulated bacteria in the floc. The flocs and associated oil particles may only act as reservoirs of substrate for free-living bacteria, which may move freely in and out of flocs. It seems clear, nevertheless, that in Patricia Bay the aggregation of finely dispersed crude oil is a sufficient, if not necessary, prerequisite for biodegradation to take place. Although the involvement of bioflocs has not been specifically addressed in other mesocosm experiments, an extensive body of literature exists that examines the propensity of bacteria to self-aggregate or attach to surfaces through the formation of exocellular adhesive polymers. Indeed, recent work by Biddanda (1985, 1986) emphasizes the importance of bacterial aggregation in carbon and energy flow and establishes that certain conversions of dissolved organic carbon (DOC) to particulate organic carbon (POC) that had formerly been considered abiological processes were actually mediated by microorganisms. Thus, it seems that attempting to describe the phenomenon of floc formation in the Patricia Bay mesocosm experiments in the context of microbial biodegradation of oil is too restrictive. In an expanded view, petroleum oil dispersions of a size class that may be considered as POC or DOC in the classical view (i.e., passing through or retained by a 0.45-μm filter) may be thought of as being a potentially exploitable carbon source by bacterial populations that are predisposed to collect or stick to it by prevailing environmental conditions. The appearance of a floc will depend on the size of the carbon source. The appearance may vary from a biofilm on a very large substrate structure to an amorphous agglomeration in the case of micron- and submicron-sized substrate particles.

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Alldredge, A.L., Cohen, Y. 1987. Can microscale chemical patches persist in the sea? Microelectrode study of marine snow, fecal pellets. Science, 235, 689–691.

Asper, V.L. 1987. Measuring the flux and sinking speed of marine snow aggregates. Deep-Sea Research, 3, 1–17.

Atkinson, B., Rahman, F.-Ur. 1979. Effect of diffusion limitations and floc size distributions on fermenter performance and the interpretation of experimental data. Biotechnology and Bioengineering, 21, 221–251.

Benefield, L., Molz, F. 1983. A kinetic model for the activated sludge process which considers diffusion and reaction in the microbial floc. Biotechnology and Bioengineering, 25, 2 591–2 615.

Biddanda, B.A. 1985. Microbial synthesis of macroparticulate matter. Marine Ecology, Progress Series, 20, 241–251.

______ 1986. Structure and function of marine microbial aggregates. Oceanologica Acta, 9, 209–211.

Cameotra, S.S., Singh, H.D., Hazarika, A.K., Baruah, J.N. 1983. Mode of uptake of insoluble solid substrates by microorganisms: II — Uptake of solid n-alkanes by yeast and bacterial species. Biotechnology and Bioengineering, 25, 2 945–2 956.

Canevari, G.P., Cranford, N.J. 1974. United States Patent 3,793,218, issued 19 February 1974. US Government Patent Office, Washington, DC, USA.

Cretney, W.J., Macdonald, R.W., Wong, C.S., Green, D.R., Whitehouse, B., Geesey, G.G. 1981. Biodegradation of a chemically dispersed crude oil. *In* Proceedings of the 1981 Oil Spill Conference — Prevention, Behavior, Control, Cleanup. American Petroleum Institute, Washington, DC, USA. Publication 4334, 37–43.

Csanady, G.T. 1986. Mass transfer to and from small particles at sea. Limnology and Oceanography, 31, 237–248.

Escher, A., Characklis, W.G. 1982. Algal–bacterial interactions within aggregates. Biotechnology and Bioengineering, 24, 2 283–2 290.

Fieser, L.F., Fieser, M. 1961. Advanced organic chemistry. Reinhold Publishing, New York, NY, USA. 1158 pp.

Foght, J.M., Westlake, D.W.S. 1982. Effect of the dispersant Corexit 9527 on the microbial degradation of Prudhoe Bay oil. Canadian Journal of Microbiology, 28, 117–122.

Gearing, J.N., Gearing, P.J., Wade, T., Quinn, J.G., McCarty, H.B., Farington, J., Lee, R.F. 1979. The rates of transport and fates of petroleum hydrocarbons in a controlled marine ecosystem, and a note on analytical variability. *In* Proceedings of the 1979 Oil Spill Conference — Prevention, Behavior, Control, Cleanup. American Petroleum Institute, Washington, DC, USA. Publication 4308.

Gearing, P.J., Gearing, J.N. 1982. Transport of No. 2 fuel oil between water column, surface microlayer and atmosphere in controlled ecosystems. Marine Environmental Research, 6, 133–143.

Glasstone, S., Lewis, D. 1960. Elements of physical chemistry. Van Nostrand, Princeton, NJ, USA. 758 pp.

Hagstrom, A. 1977. The fate of oil in a model ecosystem. Ambio, 6, 229–231.

Harrison, P.J., Cochlan, W.P., Acreman, J.C., Parsons, T.R., Thompson, P.A., Dovey, H.M. 1986. The effects of crude oil and Corexit 9527 on marine phytoplankton in an experimental enclosure. Marine Environmental Research, 18, 93–109.

Hebel, D., Knauer, G.A., Martin, J.H. 1986. Trace metals in large agglomerates (marine snow). Journal of Plankton Research, 8, 819–824.

Hinga, K.R., Pilson, M.E.Q., Lee, R.F., Farrington, J.W., Tjessem, K., Davis, A.C. 1980. Biogeochemistry of benzanthracene in an enclosed marine ecosystem. Environmental Science and Technology, 14, 1 136–1 143.

Hinga, K.R., Pilson, M.E.Q., Almquist, G.A., Lee, R.F. 1986. The degradation of 7,12-dimethylbenz(a)anthracene in an enclosed marine ecosystem. Marine Environmental Research, 18, 79–91.

Ho, C.S., Baddour, R.F., Wang, D.I.C. 1984. Effective diffusivity of oxygen in microbial pellets. Biotechnology Advances, 2, 21–33.

Hodson, R.E., Azam, F., Lee, R.F. 1977. Effects of four oils on marine bacterial populations: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 119–126.

Iman, S.H., Bard, R.F., Tosteson, T.R. 1984. Specificity of marine microbial surface interactions. Applied and Environmental Microbiology, 48, 833–839.

Kulkarni, K., Barnett, S.M. 1979. Microbial growth on hydrocarbons: role of flocs. Biotechnology and Bioengineering, 21, 337–339.

Laake, M., Tjessem, K., Rein, K. 1984. Fate of tritiated Ekofisk crude oil in a controlled ecosystem experiment with North Sea plankton. Environmental Science and Technology, 18, 641–647.

Lange, R. 1985. A 100 ton experimental oil spill at Halten Bank, off Norway. *In* Proceedings of the 1985 Oil Spill Conference — Prevention, Behavior, Control, Cleanup. American Petroleum Institute, Washington, DC, USA. Publication 4385, 503–505.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parsons, T.R., Lalli, C.M., Wu, J. 1985. Microbial response to crude oil and Corexit 9527: seafluxes enclosure study. Microbial Ecology, 11, 337–351.

Lee, R.F., Gardner, W.S., Anderson, J.W., Blaylock, J.W., Barwell-Clarke, J. 1978. Fate of polycylic aromatic hydrocarbons in controlled ecosystem enclosures. Environmental Science and Technology, 12, 832–838.

Lewin, R. 1984. Microbial adhesion is a sticky problem. Science, 224, 375–377.

Logan, B.E., Hunt, J.R. 1987. Advantages to microbes of growth in permeable aggregates in marine systems. Limnology and Oceanography, 32, 1034–1048.

Loosdrecht, M.C.M., Lyklema, J., Norde, W., Schraa, G., Zehnder, A.J.B. 1987. Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. Applied and Environmental Microbiology, 53, 1898–1901.

MacNeill, M.R., Goodman, R.H., Bodeux, J.B., Corry, K.E., Paddison, B.A. 1985. Dispersant tests in a wave basin. In Proceedings of the 1985 Oil Spill Conference — Prevention, Behavior, Control, Cleanup. American Petroleum Institute, Washington, DC, USA. Publication 4385, 463–469.

Mallee, F.M., Blanch, H.W. 1977. Mechanistic model for microbial growth on hydrocarbons. Biotechnology and Bioengineering, 19, 1793–1816.

Mandelbrot, B.C. 1983. The fractal geometry of nature. Freeman, New York, NY, USA. 468 pp.

Marty, D., Armand, B., Gatellier, C. 1979. Effects of three oil spill dispersants on marine bacterial populations. Marine Pollution Bulletin, 10, 285–287.

Parsons, T.R., Harrison, P.J., Acreman, J.C., Dovey, H.M., Thompson, P.A., Lalli, C.M., Lee, L., Li, G., Chen, X. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527: Part II — Biological effects. Marine Environmental Research, 13, 265–275.

Paul, J.A., Jeffrey, W.H. 1985. Evidence for separate adhesion mechanisms for hydrophilic and hydrophobic surfaces in *Vibrio proteolytica*. Applied and Environmental Microbiology, 50, 431–437.

Reddy, P.G., Singh, H.D., Roy, P.K., Baruah, J.N. 1982. Predominant role of hydrocarbon solubilization in the microbial uptake of hydrocarbons. Biotechnology and Bioengineering, 24, 1 241–1 269.

Reddy, P.G., Singh, H.D., Pathak, M.G., Bhagat, S.D., Baruah, J.N. 1983. Isolation and functional characterization of hydrocarbon emulsifying and solubilizing factors produced by a *Pseudomonas* species. Biotechnology and Bioengineering, 25, 387–401.

Shanks, A.L., Trent, J.D. 1979. Marine snow: microscale nutrient patches. Limnology and Oceanography, 24(5), 850–854.

Smetacek, V.S. 1985. Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. Marine Biology, 84, 239–251.

Swaranjit, S.C., Singh, H.D., Baruah, J.N. 1984. Demonstration of extracellular alkane solubilizing factor produced by *Endomycopsis lipolytica* Y.M. Biotechnology and Bioengineering, 26, 554–556.

Traxler, R.W., Bhattacharya, L.S. 1978. Effect of a chemical dispersant on microbial utilization of petroleum hydrocarbons. *In* McCarthy, L.T., Lindblom, G.P., Walter, H.F., ed., Chemical dispersants for the control of oil spills. American Society for Testing and Materials, Philadelphia, PA, USA. ASTM Special Technical Publication 659, 180–187.

Una, G.V., Garcia, M.J.N. 1983. Biodegradation of non-ionic dispersants in seawater. European Journal of Applied Microbiology and Biotechnology, 18, 315–319.

Vaccaro, R.F., Azam, F., Hodson, R.E. 1977. Response of natural marine bacterial population to copper: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 17–22.

Velankar, S.K., Barnett, S.M., Houstan, C.W., Thompson, A.R. 1975. Microbial growth on hydrocarbons — some experimental results. Biotechnology and Bioengineering, 17, 241–251.

Wakeham, S.G., Canuel, E.A., Doering, P.H. 1986a. Geochemistry of volatile organic compounds in seawater: mesocosm experiments with ^{14}C-model compounds. Geochimica et Cosmochimica Acta, 50, 1 163–1 172.

______ 1986b. Behaviour of aliphatic hydrocarbons in coastal seawater: mesocosm experiments with [^{14}C]octadecane and [^{14}C]decane. Environmental Science and Technology, 20, 574–580.

Wells, P.G., Abernethy, S., Mackay, D. 1985. Acute toxicity of solvents and surfactants of dispersants to two planktonic crustaceans. *In* Proceedings of the 8th Annual Arctic Marine Oilspill Program Technical Seminar, 18–20 June 1985, Edmonton, Alberta. Technical Services Branch, Environmental Protection Service, Department of the Environment, Ottawa, ON, Canada. 228–240.

Witten, T.A., Cates, M.E. 1986. Tenuous structures from disorderly growth processes. Science, 232, 1 607–1 612.

Wong, C.S., Whitney, F.A., Cretney, W.J., Lee, K., McLaughlin, F., Wu, J., Zhuang, D. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527: part I. Marine Environmental Research, 13, 247–263.

Zosim, Z., Gutnick, D., Rosenberg, E. 1982. Properties of hydrocarbon-in-water emulsions stabilized by Acinetobacter RAG-1 emulsan. Biotechnology and Bioengineering, 24, 281–292.

Microbial Degradation of Petroleum in an Intertidal Beach Environment – In Situ Sediment Enclosure Studies

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Chemical and bacteriological data obtained from in situ sediment enclosure experiments conducted in the intertidal zone of a sand beach in Atlantic Canada demonstrated that increases in bacterial numbers, after the addition of an "unweathered" light crude oil (a condensate), may not be a direct result of oil utilization. Although biodegradation of condensate stranded in intertidal sediments may be limited by nutrient availability, sustained enhancement of biodegradation rates was not observed immediately after the addition of an oleophilic nutrient mixture. Limited enhancement of oil biodegradation was observed in sediments seeded with commercially available strains of oil-degrading bacteria. Time-series data over a 100-d period, however, showed that such allochthonous microorganisms were not sustained under natural environmental conditions.

The mechanism of diauxic growth by indigenous microorganisms appears to be a dominant factor controlling microbial responses to petroleum. Results of this study support the validity of using "open," in situ enclosure systems for studies on the fate of oil stranded within intertidal sediments and on the development and evaluation of potential methods of cleaning up oil spills.

Microorganisms with the ability to metabolize a wide range of oil components are widely distributed in nature (Mulkins-Phillips and Stewart 1974; Atlas 1981). Biodegradation has been recognized as a principal process in weathering petroleum in the marine environment (NAS 1985). Although the potential capability of indigenous marine microflora to degrade oil is a function of the physicochemical properties of seawater and oil, the environmental conditions, and the biota, it is now generally accepted that nutrient availability is the most common limiting factor (Atlas and Bartha 1972).

The optimum ratios of carbon, nitrogen, and phosphorus required to support maximum biodegradation rates for oil have been defined in the laboratory (Bridie and Bos 1971), and experimental studies have shown that fertilization with nitrogen and phosphorus offer great promise as a countermeasure for combatting oil spills in the marine environment (Atlas and Bartha 1972, 1973; Walker et al. 1976; Lee and Levy 1989). Under actual field conditions, however, water-soluble nutrients are rapidly diluted into the surrounding waters; thus, they are removed from the oil–water interface where biodegradation occurs. To overcome this problem, oleophilic nutrients that maintain optimum concentrations of nitrogen and phosphorus at the oil–water interface have been developed. Results of preliminary tests with water-soluble nutrients encapsulated in paraffin (Atlas and Bartha 1973; Olivieri et al. 1976) or microemulsions of water-soluble nutrients in a lipophilic phase (Tramier and Sirvins 1983; Sirvins and Angles 1986) have been encouraging.

Although some microorganisms with constitutive enzyme systems to degrade oil have been isolated, most have adaptive systems that appear only after a period of contact with hydrocarbons. Even though some toxic fractions of the oil may be lost during this period, physicochemical reactions may also produce components that are highly toxic or refractile. Seeding with specific strains of oil-degrading bacteria immediately after an oil spill may be a practical means to counteract such interactions.

In recent years, substantial reserves of natural gas and condensate have been found in the Scotian Shelf off the coast of Nova Scotia, Canada. Chemical analysis of Scotian Shelf condensate (SSC), a liquid of low viscosity and high volatility (Strain 1986), has demonstrated that it contains a high proportion of low molecular weight hydrocarbons, some of which are known to be highly toxic to marine biota (Anderson et al. 1974; Calder and Lader 1976). Thus, as a consequence of offshore oil exploration and transport, the probability exists that condensate could be spilled in the future. To date, it has been assumed that spills of SSC would have little environmental impact and that cleanup could be left to rapid natural processes. These assumptions were based on studies by Ross (1982) and Hutcheson et al. (1983), which predicted that 65% of a surface SSC slick would evaporate within 1 h and that dispersions of condensate in seawater would weather within hours. However, contradictions to these observations have also been noted. For example, Blumer et al. (1973) reported no changes in the composition of No. 2 fuel oil buried in sediments 6 months after a spill at West Falmouth, Massachusetts, and Strain (1986) demonstrated that even the very light components of SSC, such as C_7 and C_8 acyclic and cyclic saturated hydrocarbons, could persist in the intertidal zone of sandy beaches for more than 6 months.

Low-energy systems, such as beaches and salt marshes, are considered to be among the most sensitive ecosystems and the most difficult to clean after coastal oil spills. In the present study, an attempt was made to assess the feasibility of using enhanced biodegradation by nutrient enrichment and microbial seeding to remove light crude oils, such as SSC, stranded within a low-energy sand beach in Atlantic Canada.

Materials and methods

Scotian Shelf condensate from an exploratory well in the Venture Field on the Scotian Shelf was obtained through Mobil Oil Canada Limited. INIPOL EAP 22 an oleophilic nutrient developed by Elf Aquitain, was obtained from CECA SA, France. This nutrient mixture is a microemulsion containing a solution of urea in brine, encapsulated in oleic acid as the external phase, with laurylether phosphate as a surfactant (Tramier and Sirvins 1983; Sirvins and Angles 1986).

Oil-degrading bacteria were obtained from Cole-Parmer Instrument Company (Cat. R245-70). This mixed culture, sold in the form of a dry granular powder consisting of bran amended with strains of *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, and *Bacillis subtilis* adapted to metabolize crude oils in seawater, was rehydrated with sterile seawater (100 g·L^{-1}) 2 h before use.

Field site

Field studies were conducted in the intertidal zone of a low-energy sand beach located in a sheltered cove on the eastern shore of Nova Scotia, Canada (Fig. 1). Sediment at the site is composed of well-sorted, medium-fine sand (250 μm diameter) low in organic carbon (Strain 1986). Visual observation of a distinct black layer in sediment cores indicated that oxygenated water penetrated to about 1 m depth within the intertidal zone of the beach. During the experiments, surface seawater temperature at the site ranged from 11.5 to 19.5°C, and nutrient concentrations from 0.02 to 1.50 μmol·L^{-1} for phosphate and 0.38 to 2.53 μmol·L^{-1} for nitrate.

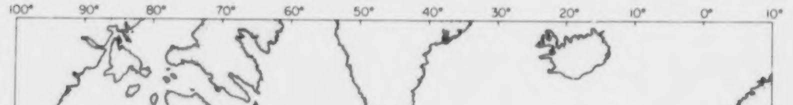

Fig. 1. Location of field site on the eastern shore of Nova Scotia, Canada.

Field procedures

Perforated stainless steel trays (28 cm long × 23 cm wide × 10 cm deep with 6-mm holes on 25-mm centres) and Nitex bags (264 μm mesh) of similar dimensions were used as in situ enclosures to study the effect of bacterial seeding and nutrient enrichment on biodegradation of SSC stranded in the intertidal zone under natural conditions. The enclosures, filled with untreated sand (collected from the surrounding area) and treated sand (combinations of 20 or 200 mL of SSC, 20 mL of INIPOL EAP-22, and 100 mL of rehydrated bacterial culture, mixed with 7 L of wet sand), were buried flush with the existing beach surface (secured to wooden stakes spaced 1.7 m apart), in a line midway between the high and low water marks, on 10 September 1984 (experiment 1) and 16 May (experiment 2).

Subsamples of sediments recovered during the experiment were frozen (-15°C) for analysis of nutrients and oil composition as well as maintained at ambient temperature for immediate microbiological processing.

Microbial analysis

Numbers of heterotrophic and oleoclastic or petroleum-degrading bacteria in the sediment were determined by a five-tube most probable number (MPN) procedure (APHA 1981) using Difco Marine Broth (for heterotrophs) and saline Difco Bushnell-Haas Broth amended with 1% (vol/vol) SSC (for oleoclastic bacteria with the potential to degrade condensate). After 4 weeks of incubation at the ambient surface seawater temperature recorded at the time of sample collection, the tubes were examined for bacterial growth on the basis of turbidity.

Comparison of hydrocarbon degradation rates in sediments within the experimental enclosures was accomplished by measuring the respiration rate of added ^{14}C-labeled hexadecane. Triplicate sediment samples (1.0 mL) resuspended in 10 mL of filtered seawater (0.22 μm Millipore) with 0.1 μCi (3700 Bq) of added n-[1-^{14}C] hexadecane (60 $mCi \cdot mmol^{-1}$ (2.22 $GBq \cdot mmol^{-1}$)) were incubated at ambient seasonal temperatures in 25-mL centre-well incubation flasks (Kontes Scientific Glassware). After 24 h of incubation, 1 mL of 2 *N* H_2SO_4 was added to the incubation flasks to halt biological activity and to drive off dissolved CO_2. The CO_2 adsorbent, β-phenethylamine (0.1 mL) was injected onto accordion-folded, 25-mm diameter Whatman GF/C glass-fibre filters suspended in the centre well of the flasks. The phenethylamine-treated filters were recovered after 12 h and placed in 20 mL of Beckman HP/b scintillation cocktail before determination of radioactivity with a Beckman LS-3801 liquid scintillator. Filter blank values were obtained by acidifying a set of samples with H_2SO_4 before isotope addition. The turnover time of hexadecane in individual samples (based on respiration rates) was calculated using actual hexadecane values found in the sediments (determined by gas chromatography).

Chemical analysis

Concentrations of orthophosphate and nitrate in surface water samples were measured using a Technicon Autoanalyzer II (TIS 1972, 1973). Concentrations of total nitrogen in sediment samples were measured using a modification of the method described by Shepherd and Davies (1981). Aliquots of sediment (0.2 g) were added to 4.5 mL of distilled water and 1.5 mL of alkaline peroxodisulfate reagent, sealed in 10-mL ampoules, and autoclaved for 30 min to convert the organic nitrogen to nitrate. After dissolution of the hydroxide precipitate in the ampoules with HCl and dilution with tris buffer, total nitrogen was determined as nitrate using the Autoanalyzer.

The total concentration of oil in the sediment ($\mu g \cdot g^{-1}$ of sediment) was measured by infrared and fluorescence analyses. For the former, 10 g of sediment were extracted for 1 min with CCl_4 in a high-speed blender. The extract was filtered and diluted to within a 0–100 ppm range and quantified by comparing the intensity of the C–H stretching frequency at 2930 cm^{-1} with that of reference solutions of SSC in CCl_4 using a Beckman Acculab 6 infrared spectrophotometer. For fluorescence analysis, sediment samples (0.2 g) were extracted with 50 mL of hexane for 2 min. A 1 mL aliquot of this extract was diluted to 25 mL with hexane and the total concentration of SSC was determined by comparing the intensity of emission at 324.5 nm (excitation 280 nm) with that of a series of SSC solutions of known concentrations using a Perkin-Elmer MPF-441B fluorescence spectrophotometer.

Changes in the composition of SSC in the field samples were monitored by capillary gas chromatography. Samples of wet sediment (0.5–1.0 g) were extracted with 2.0 mL of Resi-analyzed hexane and dried with 0.5 g of anhydrous Na_2SO_4. A small amount of the extract (0.5–0.6 μL) was injected (cool on column) into a Hewlett-Packard 5880A gas chromatograph with a 15-m DB-1 0.25-μm fused silica capillary column. Conditions for chromatography were as follows: temperature program, 60–265°C at $60°C \cdot min^{-1}$; FID, 265°C; and He flow, 1.5 $mL \cdot min^{-1}$. To determine the concentration of specific compounds, standards with known quantities of selected alkanes and aromatic compounds were prepared to determine instrument response factors. The ratio of n-C_{17} to pristane, the concentration of hexadecane, and total oil (based on the concentration of n-C_{19}) were calculated from the analytical results.

Results and discussion

Experiment 1

Because of insufficient dilutions during the MPN tests before day 44, numbers of heterotrophic bacteria in the sediments during this period were not determined. A gradual increase in the abundance of heterotrophic bacteria was observed between days 44 and 100 in the untreated "control" tray (Fig. 2). In comparison to the untreated "control," significant enrichment of heterotrophic bacteria was observed on day 44 in other trays. Although nutrient enrichment, bacterial seeding, or both enhanced the numbers of heterotrophic bacteria in unoiled sediments on day 44, observations on days 60 and 100 suggest that this enhancement was not sustained. There was no evidence in condensate-treated trays that microbial seeding, nutrient enrichment, or both significantly enhanced the abundance of heterotrophic microflora between days 44 and 100.

The number of bacteria with the potential to degrade SSC in the untreated "control" tray (Fig. 3a) increased gradually during the experimental period. In the tray treated with oleophilic nutrients only, with the exception of one point (observed after 15 d), INIPOL EAP-22 did not enhance the growth of oleophilic bacteria.

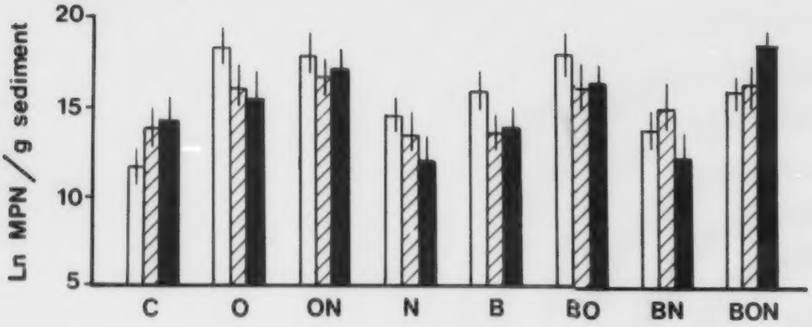

Fig. 2. Most probable number (MPN) of total heterotrophic bacteria in samples collected on day 44 (open bar), day 60 (shaded bar), and day 100 (solid bar). Experimental additions of SSC (O), INIPOL EAP-22 (N), and bacteria (B), both individually and in combination, are illustrated. C represents the untreated control. Error bars = 95% confidence limits.

However, after an adaptation period during which the most of the volatile toxic components are lost (10–20 d after oil addition in oiled trays with or without nutrient enrichment, and 10–43 d, in the oiled trays seeded with bacteria), exponential increases in the number of oil-degrading bacteria were observed (Fig. 3). In contrast to expectations based on previous laboratory results (Lee and Levy 1987), enrichment of oiled sediment with the oleophilic nutrient mixture did not increase the number of oil-degrading bacteria significantly during the first 60 d of the experiment.

Similarly, microbial seeding of unoiled sediment, with or without the addition of oleophilic nutrients had no significant effect on the abundance of oil-degrading bacteria (Fig. 3). Presumably, this is because the bacterial culture was unable to compete with indigenous microflora. In oiled sediments that were seeded, on the other hand, an increase in the MPN of oil-degrading bacteria was observed after a 15-d induction period. In contrast with sediment treated with SSC only, however, it appears that a different population of oil-degrading bacteria was stimulated by microbial seeding of oiled sediments because a distinctly different growth response was observed in the oleophilic bacterial population in seeded sediments (the exponential growth rate and duration of the stationary phase were reduced). Although this population was initially capable of outcompeting indigenous microorganisms in contaminated sediments, it declined rapidly in mid-November (after 60 d) in response to alterations in oil composition or a decrease in ambient temperature or both.

Analysis of total nitrogen concentration in the sediments demonstrated that the oleophilic nutrient was lost within a 2-d period (Table 1) and was, therefore, ineffective in promoting biodegradation of the condensate under actual field conditions.

Estimates of the total concentration of oil in the sediments by gas chromatography, fluorescence spectroscopy, and infrared spectroscopy (Table 2) indicated a rapid loss of condensate (71–82%) from the trays. Because of high inter- and intratray variability, resulting from tidal-driven migration of unoiled sand into and contaminated sand out of the trays, no significant difference in the rate of condensate loss in response to experimental treatment was observed among oiled trays.

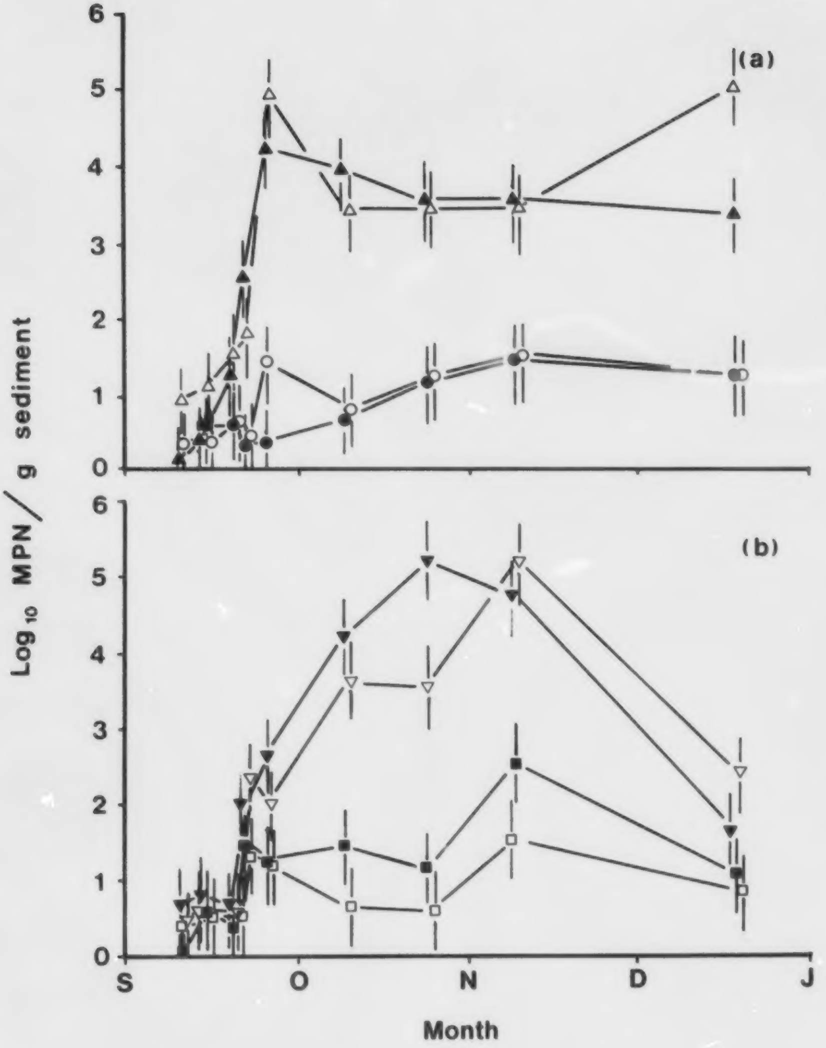

Fig. 3. Change in the most probable number (MPN) of oleophilic bacteria within in situ sediment enclosures in response to nutrient enrichment (a) and microbial seeding (b). Sediments in the enclosures were treated with the following: INIPOL EAP-22 (○); SSC (▲); SSC and INIPOL EAP-22 (△); bacteria (■); bacteria and INIPOL EAP-22 (□); bacteria and SSC (▼); and bacteria and SSC with INIPOL EAP-22 (▽). Untreated control is represented by (●). Error bars = 95% confidence limits.

Similar losses of oil from experimental sediment enclosures were reported by Anderson et al. (1983) during a study of Prudhoe Bay crude oil stranded in intertidal sediments.

Table 1. Total concentration of nitrogen in sediments (μg/g).

Day	C	O	O+N	N	B	B+O	B+N	B+O+N
0	19.5	10.5	106.0	102.0	26.5	30.0	104.5	98.5
2	19.5	15.5	10.5	17.0	18.0	21.5	18.0	18.0
4	18.0	15.0	11.0	15.0	19.0	19.5	19.0	17.0
11	17.0	13.0	13.0	14.0	20.5	20.5	21.0	19.0
29	20.0	13.5	11.0	—	25.0	25.0	17.5	34.0
60	18.5	16.5	14.0	19.0	19.5	26.0	16.0	24.0
100	18.0	19.0	18.5	18.5	—	—	—	—

Notes: Experimental treatments were SSC (O), INIPOL EAP-22 (N), and bacteria (B), both individually and in combination. C represents the untreated control.

Table 2. Total oil concentration in the sediments (μg/g).

Day	GC	IR	FL
0	18 100	7 698	16 400
4	3 229	2 180	3 220
11	4 995	4 449	6 670
29	3 660	2 308	5 000
60	4 489	2 860	4 930
100	1 136	598	1 420

Note: Analysis by gas chromatography (GC), infrared spectroscopy (IR), and fluorescence spectroscopy (FL).

Because n-alkanes are more susceptible to biodegradation than isoprenoids, a decrease in the n-C$_{17}$/pristane ratio has been used to infer biodegradation (e.g., Blumer and Sass 1972; Lee et al. 1985). Despite the observed increase in numbers of potential oil-degrading bacteria and the rapid decline in total oil and nitrogen in oiled sediments, evidence of SSC biodegradation (based on the ratio of n-C$_{17}$/pristane) was only observed in the field when experimental enclosures were seeded with bacteria (Fig. 4).

Experiment 2

To maintain the optimal nutrient concentrations required for biodegradation processes, INIPOL EAP-22 was added intermittently in the second experiment by reapplying the nutrients after each sampling period. Furthermore, as a countermeasure to tidal-driven losses of treated sediments from the enclosures, bags constructed from Nitex netting (264 μm mesh) were used as enclosures. Initial application of the oleophilic nutrient was not made until 19 d after the addition of condensate because results from experiment 1 indicated that a time period of this length was required to allow most of the toxic components in SSC to be lost via dissolution and evaporation, and to allow indigenous microflora to recover and adapt to the contaminated sediment.

Although total numbers of heterotrophic bacteria were stimulated by the addition of the oleophilic nutrient in unoiled enclosures, sustained enhancement was not observed without intermittent additions (Fig. 5a). Stimulation of heterotrophic bac-

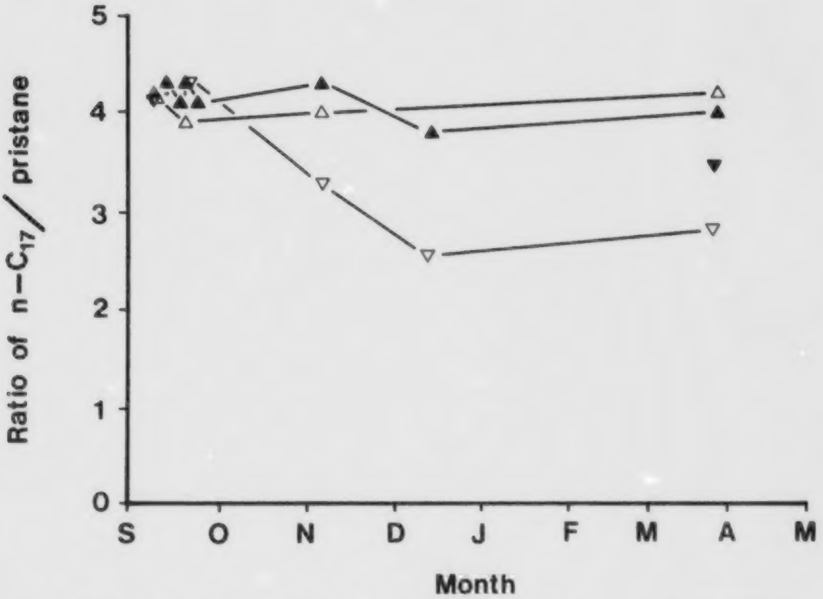

Fig. 4. Ratio of n-C_{17}/pristane within in situ enclosures at Long Cove treated with SSC (▲), SSC and INIPOL EAP-22 (△), SSC and bacteria (▼), and SSC and INIPOL EAP-22 with bacterial seeding (▽).

teria was also observed in enclosures treated with a reduced concentration of condensate (Fig. 5b); however, further enhancement of bacterial numbers was not observed after a single application of the nutrient mixture. Similarly, in enclosures treated with higher concentrations of oil (Fig. 5c), elevated numbers of heterotrophic bacteria were observed relative to untreated control sediment and a single application of the oleophilic nutrient had no significant effect. However, although intermittent addition of the oleophilic nutrient in the oiled enclosure supported higher numbers of heterotrophic bacteria (Fig. 5c), numbers were not higher than those observed in unoiled sediment that received intermittent additions of the nutrient.

The number of bacteria with the potential to degrade SSC in unoiled sediments was enriched through application of the oleophilic nutrient mixture (Fig. 6a). Although elevated numbers of oil-degrading bacteria were supported by the intermittent addition of nutrients, in contrast to heterotrophic bacteria, oil-degrading bacteria were apparently less tolerant to low temperatures and declined (in November) in response to the seasonal decrease in ambient temperature. A rapid increase in the number of condensate-degrading bacteria was observed in oiled enclosures after a 2 week lag period (Fig. 6b, c). With the exception of one sampling period in the enclosure receiving a lower concentration of condensate, application of INIPOL EAP-22 had no significant effect on numbers of oil-degrading bacteria.

Oil degradation in the sediment, as inferred by the turnover time for hexadecane in the sediments, suggests that INIPOL EAP-22 may, in fact, inhibit oil degradation

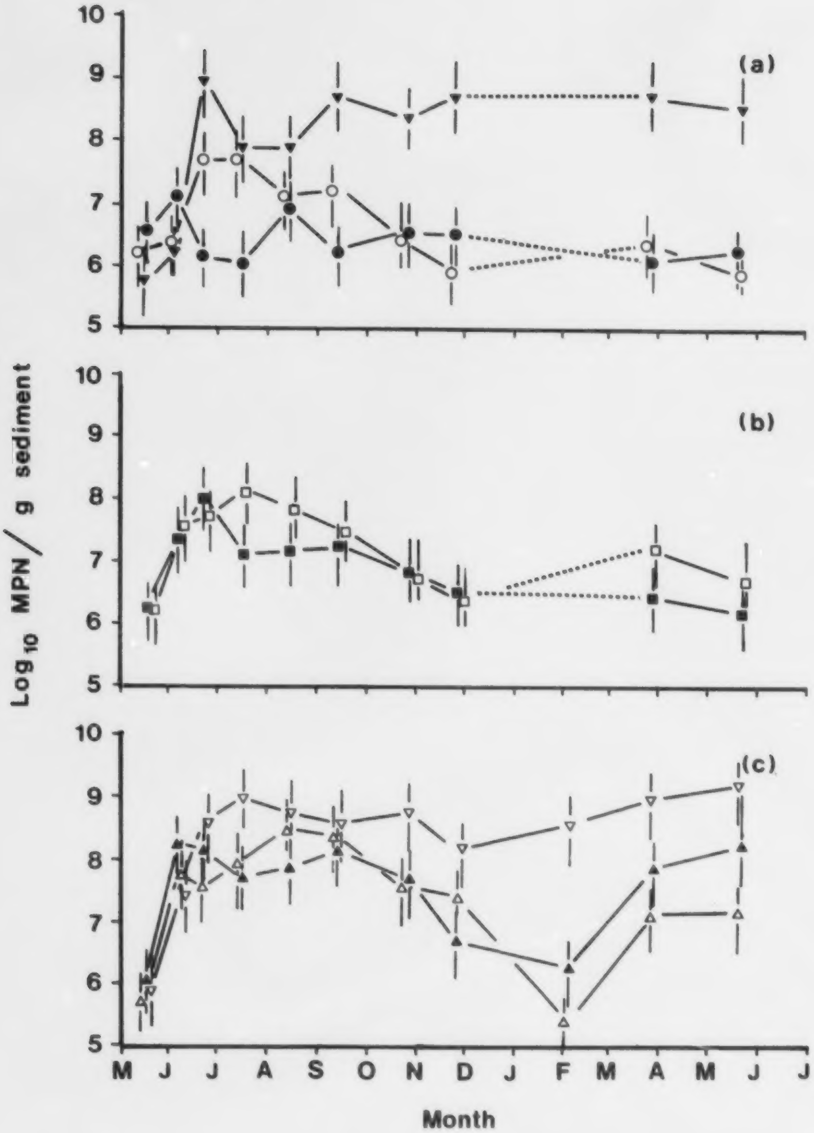

Fig. 5. Change in the most probable number (MPN) of total heterotrophic bacteria in response to SSC and nutrient enrichment. Treatment of in situ enclosures containing 7 L of sediment was as follows: (a) untreated "control" (●), single application of INIPOL EAP-22 (○), and intermittent addition of INIPOL EAP-22 (▼); (b) 20 mL SSC (■) and 20 mL SSC with a single application of INIPOL EAP-22 (□); (c) 200 mL SSC (▲), 200 mL SSC with a single application of INIPOL EAP-22 (△), and 200 mL SSC with intermittent addition of INIPOL EAP-22 (▽).
Error bars = 95% confidence limits.

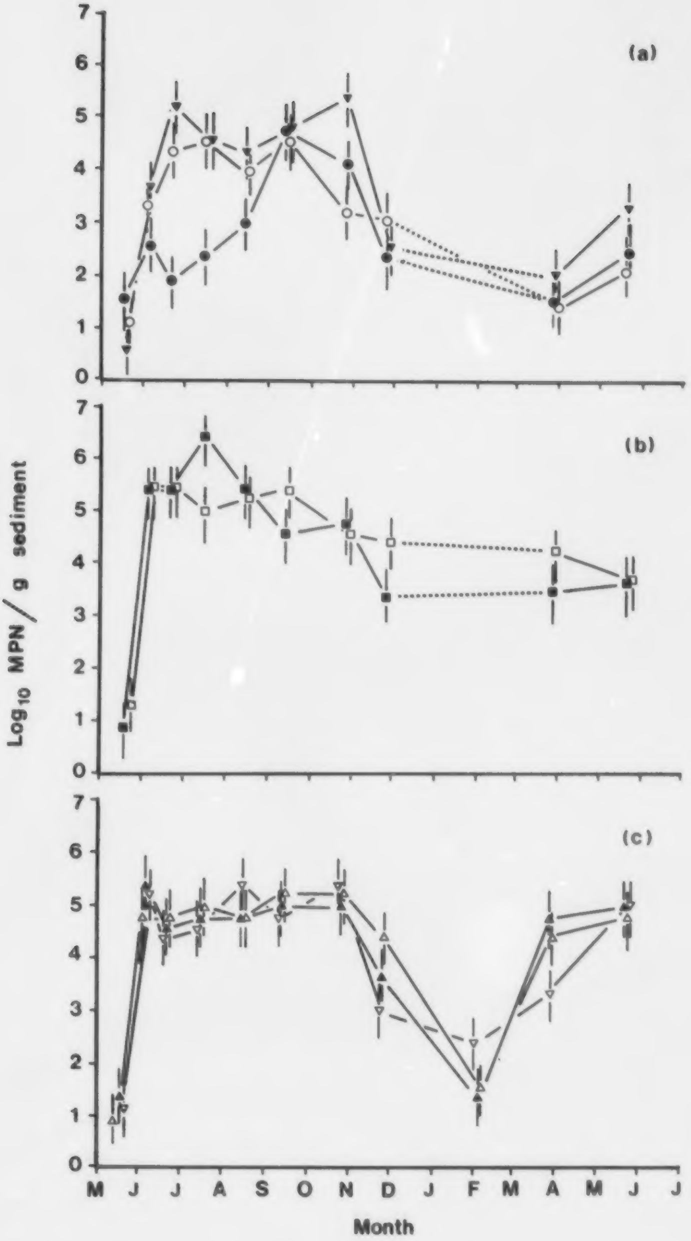

Fig. 6. Change in the most probable number (MPN) of oleophilic bacteria in response to SSC and nutrient enrichment.
Treatment of sediment in enclosures as described in Fig. 5.

(Fig. 7). In oiled enclosures that were similarly treated, the turnover time of hexadecane demonstrated that both single and intermittent additions of the oleophilic nutrient mixture suppressed biodegradation of hexadecane. In enclosures receiving an intermittent addition of nutrients, the turnover time of hexadecane was signifi-

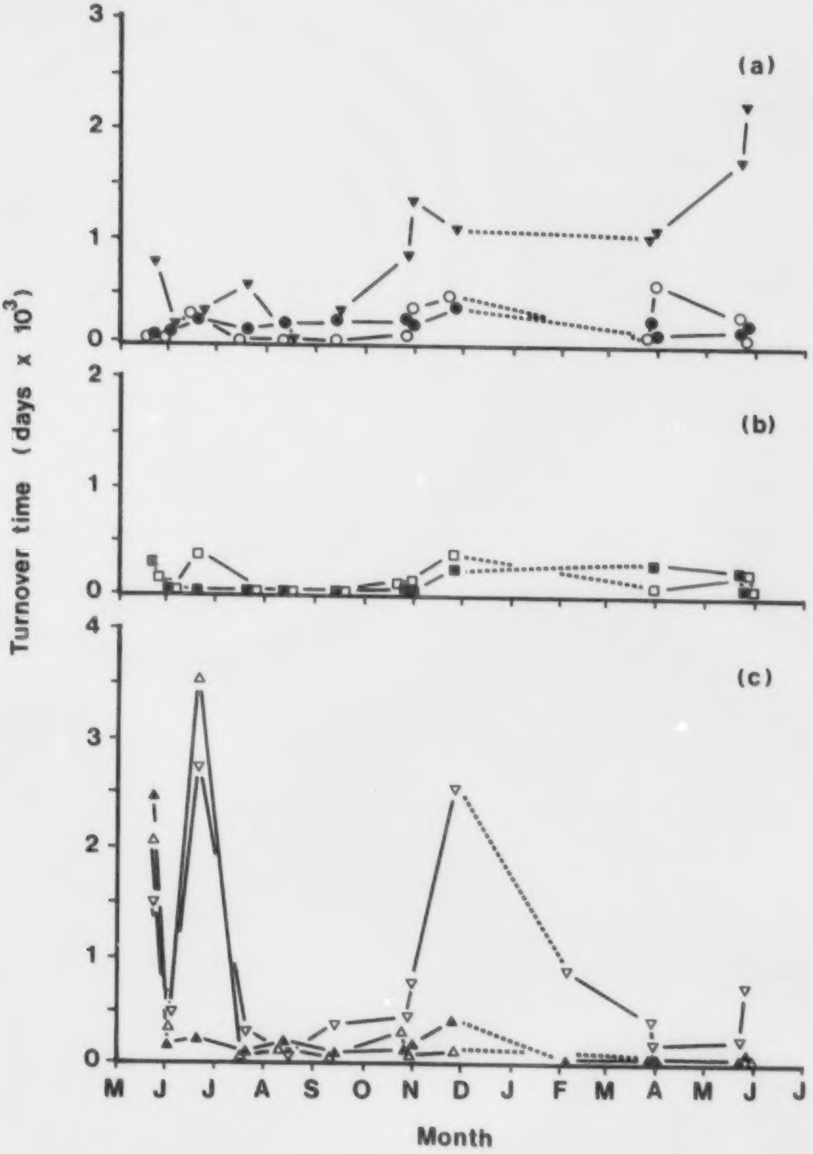

Fig. 7. Turnover time for hexadecane in the sediment in response to SSC and nutrient enrichment. Treatment of sediment in enclosures as described in Fig. 5.

cantly prolonged during the winter months because of an increase in retention of the oleophilic nutrient in the sediment and a reduction in microbial activity at lower temperatures. The observed decline in hexadecane use by indigenous microbiota in response to the oleophilic nutrient can be attributed to a diauxic growth response in which the organic nutrient mixture was preferentially used over hexadecane. This response was also apparent in biodegradation indices. For example, in the enclosure that received a reduced concentration of condensate, a single application of oleophilic nutrient interrupted the decline in the ratio of n-C_{17}/pristane (Fig. 8a). At higher oil concentrations (Fig. 8b), the n-C_{17}/pristane biodegradation index indicated that intermittent addition of the oleophilic nutrient mixture over the duration of the experiment had little effect on enhancing the natural biodegradation rate of condensate. In contrast to experiment 1, however, some enhancement in biodegradation of SSC was observed 2 months after a single application of the nutrient mixture. This enhancement was attributed to a diauxic response by the population of bacteria, which had previously been sustained by the nutrient. Because of differences in ambient temperature, such an effect, 2 months after a single application of the nutrient, was not observed in experiment 1.

Field observations from this study differ from experimental results of Sirvins and Angles (1986) and Tramier and Sirvins (1983), which showed that the rate of oil loss in seawater, within a 7-d period, could be increased from 10–20% to 60–85%, even at temperatures as low as 3°C, by applying a similar oleophilic nutrient mixture to enhance microbial activity. Their results may be attributed to the fact that the medium crude oil used in their studies was less toxic than the condensate used in the present study or that the bacteria used in their laboratory studies were previously adapted to pure hydrocarbons and crude oil. It is likely, however, that

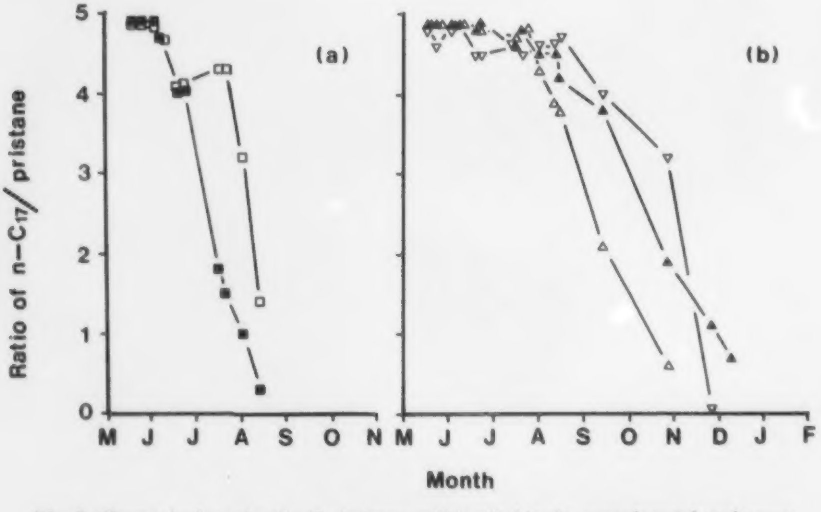

Fig. 8. Change in the ratio of n-C_{17}/pristane within oiled in situ experimental enclosures containing 7 L of sediment treated as followed: (a) 20 mL SSC (■) and 20 mL SSC with a single application of INIPOL EAP-22 (□), and (b) 200 mL SSC (▲), 200 mL SSC with a single application of INIPOL EAP-22 (△), and 200 mL SSC with intermittent addition of INIPOL EAP-22 (▽).

their field trials with natural microflora were conducted in "closed systems" in which nutrient losses were prevented because water movement and exchange in their enclosures was greatly reduced in comparison with that occurring in the natural environment. Similarly, successful demonstration of enhanced biodegradation of oil stranded on vegetation in the supralittoral zone by Halmo (1985), using an oleophilic nutrient, may be attributed to the fact that the contaminant oil was an emulsion of "weathered" crude oil from which toxic volatile components, if any, had been removed. In addition, at Halmo's study site, movement of the oleophilic nutrient from the supralittoral zone was obviously slow as application of inorganic nutrient salts was found to be just as effective in promoting biodegradation.

Conclusions

In the natural environment, numbers of microorganisms with the capacity to tolerate and degrade oil have generally been shown to increase after exposure to hydrocarbons. Such adaption by the indigenous microbial community, which results in an increase in potential rates of oil degradation, probably involves a multitude of mechanisms including gene transfer or mutation, enzyme induction, and population changes. It must be stressed, however, that these organisms may preferentially metabolize organic substrates other than hydrocarbons directly associated with the oil spill. After an oil spill, these substrates may include extracellular products, whose production may be stimulated by the oil (Lee et al. 1985), as well as organic compounds, such as nutrient supplements (e.g., INIPOL EAP-22) and oil dispersants added during active "cleanup" of the spill. Results from the present study indicate that it is not sufficient to show that organisms capable of oil degradation are present in the environment, but it is essential to demonstrate that they are, in fact, active.

The present experiment demonstrated that biodegradation of light oils, such as SSC stranded in sediments of low-energy intertidal sites in north-temperate environments, is a slow process and that only limited enhancement could be achieved through nutrient enrichment with the oleophilic nutrient mixture INIPOL EAP-22. However, laboratory experiments conclusively demonstrate that the potential for much higher rates of degradation exists (Lee and Levy 1987). Further field trials using "open," in situ, enclosure systems are required to determine the optimum method of enhancing the biodegradation of oil under natural conditions.

Acknowledgments

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Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., Hightower, G.M. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Marine Biology, 27, 75–88.

Anderson, J.W., Riley, R.G., Kiesser, S.L., Thomas, B.L., Fellingham, G.W. 1983. Natural weathering of oil in marine sediments: tissue contamination and growth of the littleneck clam, *Protothaca staminea*. Canadian Journal of Fisheries and Aquatic Science, 40, 70–77.

APHA (American Public Health Association). 1981. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC, USA. 773–805.

Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiological Review, 45, 180–209.

Atlas, R.M., Bartha, R. 1972. Degradation and mineralization of petroleum in seawater: limitation by nitrogen and phosphorus. Biotechnology and Bioengineering, 14, 309–317.

______ 1973. Stimulated biodegradation of oil slicks using oleophilic fertilizers. Environmental Science and Technology, 7, 538–541.

Blumer, M., Sass, J. 1972. Oil pollution: persistence and degradation of spilled fuel oil. Science, 176, 1120–1122.

Blumer, M., Ehrhard, M., Jones, J.H. 1973. The environmental fate of stranded crude oil. Deep-Sea Research, 20, 239–259.

Bridie, A.L., Bos, J. 1971. Biological degradation of mineral oil in seawater. Journal of the Institute of Petroleum (London), 57, 270–277.

Calder, J.A., Lader, J.H. 1976. Effect of dissolved aromatic hydrocarbons on the growth of marine bacteria in batch culture. Applied and Environmental Microbiology, 32, 95–101.

Halmo, G. 1985. Enhanced biodegradation of oil. *In* Proceedings of the 1985 Oil Spill Conference. American Petroleum Institute, Washington, DC, USA. 531–537.

Hutcheson, M.S., Keizer, P.D., Odense, R. 1983. Chemistry and toxicity of the Venture Field condensates. Report prepared by Atlantic Oceanics Company Ltd for Mobil Oil Canada Ltd, January 1983. 33 pp.

Lee, K., Levy, E.M. 1987. Enhanced biodegradation of a light crude oil in sandy beaches. *In* Proceedings of the 1987 Oil Spill Conference. American Petroleum Institute, Washington, DC, USA. 411–416.

______ 1989. Biodegradation of petroleum in the marine environment and its enhancement. *In* Nriagu, J.O., Lakshminarayana, J.S.S., ed., Aquatic toxicology and water quality management. John Wiley and Sons, New York, NY, USA. 217–243.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parsons, T.R., Lalli, C.M., Wu, J. 1985. Microbial response to crude oil and Corexit 9527: SEAFLUXES enclosures study. Microbial Ecology, 11, 337–351.

Mulkins-Phillips, G.J., Stewart, J.E. 1974. Distribution of hydrocarbon-utilizing bacteria in northwestern Atlantic waters and coastal sediments. Canadian Journal of Microbiology, 20, 955–962.

NAS (National Academy of Sciences). 1985. Oil in the sea: inputs, fates and effects. National Academy of Sciences, Washington, DC, USA. 601 pp.

Olivieri, R., Bacchin, P., Robertiello, A., Oddo, N., Degen, L., Tonolo, A. 1976. Microbial degradation of oil spills enhanced by a slow-release fertilizer. Applied and Environmental Microbiology, 31, 629–634.

Ross, S.L. 1982. The behaviour and fate of gas and condensate spills. Report prepared for Mobil Oil Canada Ltd's Venture Gas Field development on the Scotian Shelf. 85 pp.

Shepherd, R.J., Davies, I.M. 1981. A semi-automatic alkaline peroxodisulphate method for the routine determination of total dissolved nitrogen in sea water. Analytica Chimica Acta, 130, 55–63.

Sirvins, A., Angles, M. 1986. Development and effects on the marine environment of a nutrient formula to control pollution by petroleum hydrocarbons. *In* Giam, C.S., Don, H.J.M., ed., Strategies and advanced techniques for marine pollution studies: Mediterranean Sea. Springer-Verlag, Berlin, Germany. NATO ASI Series G9, 357–404.

Strain, P.M. 1986. The persistence and mobility of a light crude oil in a sandy beach. Marine Environmental Research, 19, 49–76.

TIS (Technicon Industrial Systems). 1972. Nitrate and nitrite in water and seawater. Technicon Industrial Systems, Tarrytown, NY, USA. Industrial method 158-71W, 3 pp.

______ 1973. Ortho phosphate in water and seawater. Technicon Industrial Systems, Tarrytown, NY, USA. Industrial method 155-71W, 4 pp.

Tramier, B., Sirvins, A. 1983. Enhanced oil biodegradation: a new operations tool to control oil spills. *In* Proceedings of the 1983 Oil Spill Conference. American Petroleum Institute, Washington, DC, USA. 115–119.

Walker, J.D., Colwell, R.R., Petrakis, L. 1976. Biodegradation rates of components of petroleum. Canadian Journal of Microbiology, 22, 1 209–1 213.

Part III

China–Canada MEEE Experiments

A. Sediments and Heavy Metals

Introduction to the Xiamen Marine Ecosystem Enclosed Experiments

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From 1985 to 1986, two types of experimental ecosystem enclosures were set up at the Third Institute of Oceanography, State Oceanic Administration, Xiamen, People's Republic of China, under the International Development Research Centre's cooperative program between China and Canada. The enclosures were modifications of the SEAFLUX system used at the Institute of Ocean Sciences, Sidney, BC, Canada, in which a plastic bag is used to enclose a column of seawater. The first type of plastic bag enclosure was 2 m in diameter and 4 m deep (with a volume of 10 m^3). It was placed in a stone-walled tank 20 m × 10 m × 5 m deep. The second enclosure was used in the field and consisted of bags 2 m diameter by 6 m deep containing 14 m^3 of water suspended from a catamaran moored in the eastern part of Xiamen Bay (24°32′ N, 119°11′ E), where a background investigation was conducted to compare ecological processes in the enclosures and in the open sea. Three pollution experiments were carried out in 2 years. The pollutants used were a mixture of heavy metals (Cu, Co, Hg, Pb, and Zn) at parts per billion levels in seawater, harbour sediments at parts per million levels, and petroleum at parts per million levels. Results indicated that: first, ecological processes in the enclosures and in the sea showed similarities and discrepancies; second, the ecosystem was initially suppressed by pollutants and but recovered after several days; and, third, certain species of plankton were sensitive to the pollutants. Improvements to Xiamen marine ecosystem enclosed experiment (MEEE) facilities are also discussed.

Several types of marine enclosures have been developed over the past 20 years (Menzel and Case 1977; Davies and Gamble 1979; Grice and Reeve 1982) to gain insight into marine ecosystem processes and their suppression by pollutants. Enclosure designs can be divided into four categories: land-based tanks, in-situ tanks, in-situ plastic bags, and remote lagoons. Each design has its advantages and disadvantages for ecological and chemical experiments, depending on the purpose of the experiment.

Land-based tanks, represented by the Marine Ecosystems Research Laboratory (MERL) facility in Rhode Island, USA (Pilson 1978), allow the researcher to conduct more controlled experiments with replication, and they are also less expensive than field-research facilities. The most popular marine enclosure is the in-situ plastic bag, represented by the Controlled Ecosystem Pollution Experiment (CEPEX) (Menzel and Case 1977) (now called SEAFLUX) of the Institute of Ocean Sciences of Canada.

During the last two decades, few marine enclosure experiments have been conducted in the tropics or subtropics where the biotic regime is quite different from that in temperate areas. Tropical or subtropical waters are characterized by high water temperatures, intense sunlight, and biological diversity.

With funding from the State Oceanic Administration (SOA) of China and the International Development Research Centre (IDRC), Canada, the Third Institute of Oceanography of SOA, Xiamen, People's Republic of China, was responsible for organizing the Marine Ecosystem Enclosure Experiment (MEEE) research program. More than 50 people were associated with the program in 1985 and 1986. Research scientists involved came from the Third Institute of Oceanography of SOA, Shandong College of Oceanology, Xiamen University, and the Institute of Marine Environmental Protection of SOA, People's Republic of China; and the Institute of Ocean Sciences and the University of British Columbia, Canada. In addition, many visitors from other universities and institutes in China came to visit MEEE at Xiamen when experiments were in progress in 1985 and 1986. In this paper, the facilities and general results of pollution experiments carried out in 1985 and 1986 at the Third Institute of Oceanography of SOA are presented.

Facilities and experiments

MEEE was conducted in Xiamen Bay (Fig. 1) in the southeast of China. Xiamen Bay was selected as the MEEE cooperative site for the following four reasons :

- It is a subtropical area with characteristically warm water and the ecology of the area has been well studied;
- The area is sheltered by a series of outskirt islands beyond the bay, has relatively low current velocities, and escapes major storms;
- The maximum water depth in Xiamen Bay is around 30 m and the euphotic zone varies between 5 and 6 m; and
- The experimental site is relatively remote from sources of pollution.

Facilities at Xiamen MEEE are of two types. First, in 1985, a small plastic-bag enclosure was set up in a granite tank. In this facility, two pollution experiments were carried out. The pollutants were a mixture of heavy metals and sediment taken from a discharge area in Xiamen's industrial area. Second, in 1986, a larger enclosure was set up in the eastern part of Xiamen Bay using a catamaran as a flotation device to help withstand local tidal currents and large waves. An experiment was conducted with Shengli crude oil and a chemical dispersant (Corexit 9527). Degradation of the oil was traced by ^{14}C-labeled hexadecane.

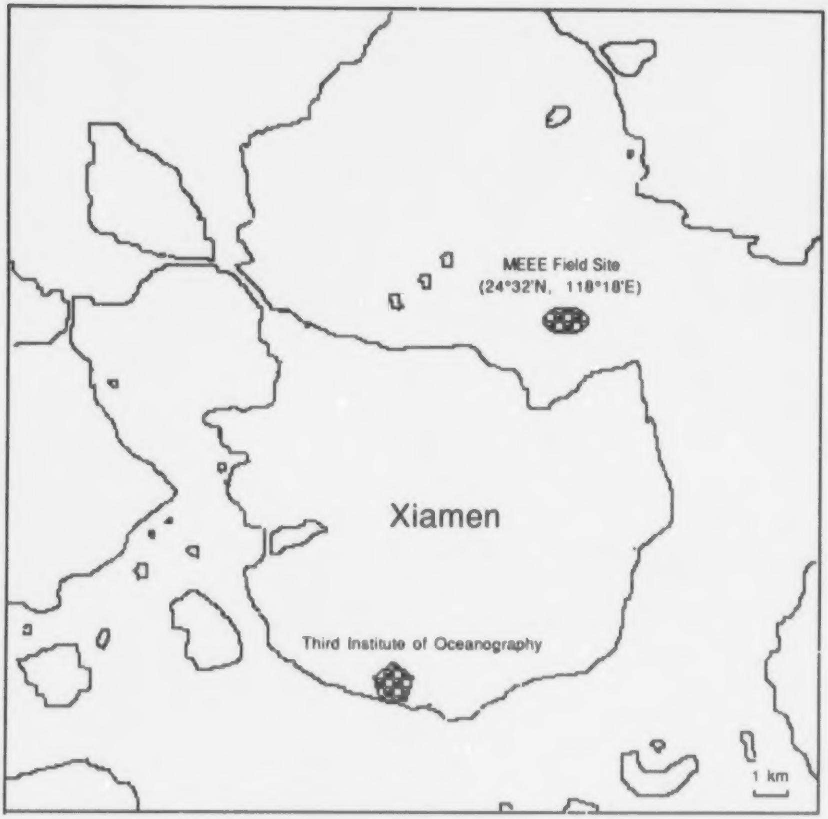

Fig. 1. Xiamen Bay and MEEE sites.

Bags

Bags were made of woven polyethylene coated with a plastic film on the inside, which made them strong and watertight, and provided a smooth surface that was in contact with seawater. A bag consisted of an upper cylinder and a lower cone, and a sediment collector tightly fixed at the bottom for collecting settled materials. A tube connected to the collector with an opening on the surface made it possible to sample settled material.

In-tank marine ecosystem enclosure (MEE)

Nine wooden floating modules were placed in a large rectangular tank (20 m × 10 m × 5 m deep) located at 24° 26′ 14″ N, 118° 5′ 25″ S on the shore of the Third Institute of Oceanography from 17 May to 5 June 1985. A fibreglass roof covered the tank to reduce the intensity of the sunlight and prevent contamination from rain (Fig. 2). The water in the tank was seawater from the bay circulated at a rate of

Fig. 2. In-tank enclosure.

60 m^3·h^{-1} during high tide (about 4 h) to keep the water temperature stable. The bags were assembled on floating wooden modules and filled simultaneously using a diaphragm pump. The seawater was taken 150 m offshore at a depth of 3 m from the surface during high tide.

Field MEE

From 23 May to 6 June 1986, a catamaran, 13 m long × 4.5 m wide, made of steel and painted with nontoxic paint, was constructed at 31° 30″ N, 118° 11′ 18″ E, where the water depth varies between 15 and 20 m, with a maximum local current of 1.5 m·s^{-1} in the fall. Four double-layered polyethylene bags were launched and filled and attached to the catamaran (Fig. 3). To ensure that the bags were stable and retained their circular shape under potentially rough weather conditions, a second steel ring was inserted outside the bag (Fig. 4) at a depth of 2.5 m and nine weights, each weighing 32 kg, were attached to the two rings (the top surface ring and the underwater ring). The cylindrical part of the bag was wrapped with a third layer of plastic to reduce the force of the current on the bag.

To fill a bag with seawater, it is first lowered a depth of 5 m, keeping the opening horizontal for a few minutes. It is then pulled up to the surface vertically as fast as possible. The water captured amounts to about 95% of the bag's volume.

Experiments

Nutrients were added to the captured water at a ratio (by atoms) of 10:10:1 N:Si:P in 1985 and 30:10:1 in 1986. On the day following, referred to as day 0 of the experiment, the nutrient additions and background pollutant levels were sampled. A few hours after background sampling, pollutants were added homogeneously from 0 to 3 m, and a water sample was taken to determine initial

Fig. 3. Field enclosure.

concentrations. Routine sampling then began and continued for about 3 weeks at 1-, 2-, or 3-d intervals depending on the pollutants added and ecological processes being studied.

Integrated seawater samples were pumped out using a peristaltic pump into a 20-L soft polyethylene "cubitainer" by slowly lowering the pump intake through the water column at a constant rate from the surface to 3 m. The seawater in the cubitainer was then subsampled for various chemical and biological determinations. Zooplankton were collected by vertical hauls from 3 m to the surface with a 202-µm nylon net 20 cm in diameter. The seawater was also sampled with a Niskin sampler at discrete depths for specific measurements.

The following chemical and biological parameters were generally determined: temperature, pH, salinity, light transmission, nutrients, chlorophyll *a*, primary production, bacterial production, bacterial numbers, phytoplankton and zooplankton identification and numbers, ratio of particulate organic carbon and nitrogen (POC/N), dissolved organic nitrogen (DON), dry weight, particle size, sinking rates, and concentration of pollutants in the water column. Detailed information on these parameters is given in MEEE group reports for 1985 and 1986. Methods used were mainly from Parsons et al. (1984).

The pollutants added to the enclosures in 1985 and 1986 are listed in Table 1. In 1985, two concentrations of heavy metals and sediments were added to the water columns of the enclosures. The concentrations of heavy metals were 10 times those of background levels in Xiamen Bay (referred to as low-level replicated treatments) and 50 times background concentrations (referred to as high-level treatments). The final concentration of sediment in the treated bags (dry weight) was 11 ppm in the low-level bag and 110 ppm in the high-level bag. In a separate enclosure, ^{65}Zn was added to trace the fate of Zn in the water column.

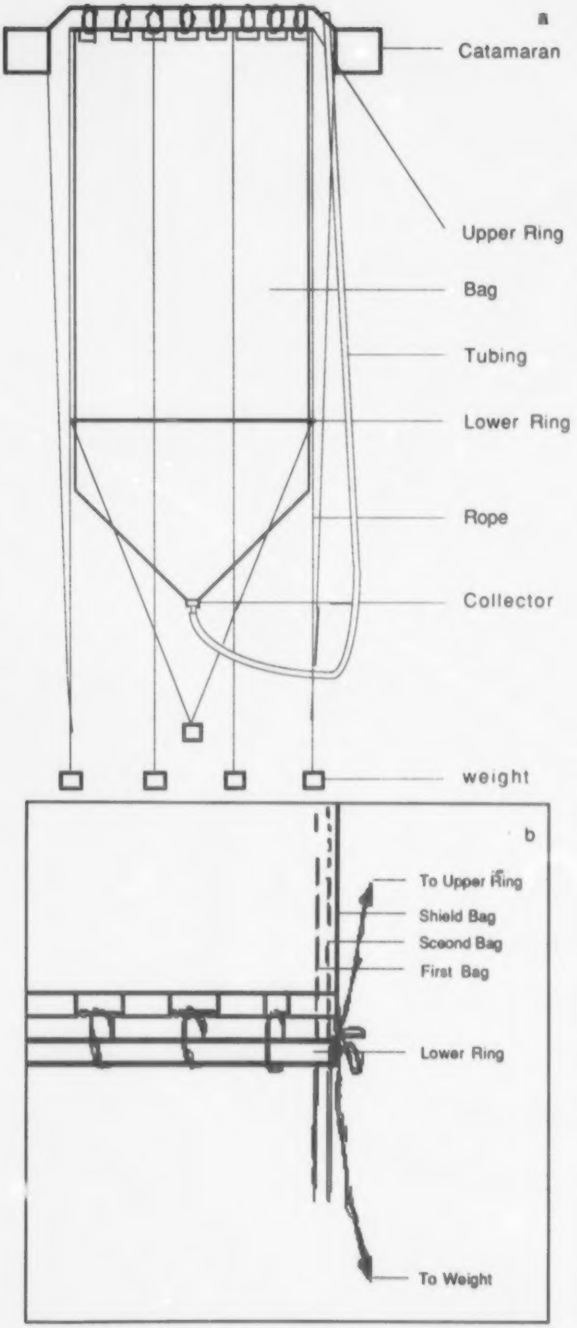
a
Catamaran
Upper Ring
Bag
Tubing
Lower Ring
Rope
Collector
weight
b
To Upper Ring
Shield Bag
Sceond Bag
First Bag
Lower Ring
To Weight

Fig. 4. In situ plastic bag (a) and ring connection (b).

Table 1. Pollutants added to enclosures.

	Background	Low level	High level
1985			
Metals (ppb)			
Cd	0.02	1.0	5.0
Cu	0.4	3.5	10.0
Pb	0.03	0.3	1.5
Zn	0.7	3.5	17.5
Hg	0.02	0.2	1.0
Sediment (ppm, dry)	—	11	110
1986			
Crude oil (ppm)	—	10	—
Corexit 9527 (ppm)	—	1	—
^{14}C hexadecane (μCi)	—	100[a]	—

[a] Equals 3.7×10^6 Bq.

In 1986, the dispersant and chemically dispersed crude oil were added homogeneously to treated enclosures from the surface to 3 m depth using a sprayer. Hexadecane (^{14}C labeled) was used to trace the fate of oil in the treated enclosures. The final concentrations were 25 mg·L^{-1} for the oil-only enclosure and 1.5 mg·L^{-1} for Corexit 9527.

Results and discussion

A comparison of ecological processes between enclosures and their environment is made and results obtained from 2 years of experiments are outlined.

Ecological parameters and processes in enclosures and in the sea

Salinity, temperature, and light intensity

For tank enclosures, salinity changes were not significant because salinity can be controlled in the tank to match the salinity in the enclosures. High light intensity, on the other hand, was a problem in the enclosure facility. In preliminary experiments conducted in 1984, an enclosure was used in the tank, but the temperature went up more than 6°C in 2 d compared with the temperature in the sea. Therefore, it was necessary to find a way to balance light intensity required for photosynthesis with an increase in temperature in the enclosures. After a discussion in the MEEE group, a roof was built over the tank to reduce the sunlight penetrating the tank by about 50% and a circulating water system was installed to cool the water in the tank with water from the sea. With these modifications, the temperature in the enclosures remained similar to that in the sea. Results of the 1985 experiment indicated that the water temperature remained stable, but photosynthesis slowed down too much because of the reduced light penetration, especially when it was cloudy. Therefore, further modifications to balance water temperature and light intensity are required.

For field enclosures, temperature and salinities inside and outside the bags were compared (Fig. 5). Unfortunately (perhaps fortunately), a major storm occurred immediately after the bags had been filled on 21 May 1986. The salinity outside the bags dropped rapidly during the first 5 d because of the heavy rain accompanying the storm. Salinity in the sea remained low until day 11 of the experiment (Fig. 5). As a result, the shape of the bag changed to that of a vase, with a smaller mouth and a large middle, exerting extra pressure on the bottom of the bag. For bag 4, after the storm, at least 15% of the enclosed water was lost and an exchange with water outside the bag may have taken place. Thus, the salinity in bag 4 was lower than in the other bags, but it was still higher than the salinity of the seawater outside the bags (Fig. 5). On day 4, the tubing used for pumping settled particulates dropped into the water, resulting in a loss of water in bag 1. From day 0 to day 11, the salinity in the bags decreased slightly, indicating that an exchange of water had occurred. On day 11, the salinity outside and inside the bags was similar. After the heavy rain at the beginning of the experiment, the weather was perfect for the remainder of the experiment. This caused the salinity in the bay to increase quickly with the exchange of coastal water.

The temperature profiles inside and outside the enclosures were almost the same because of good mixing in the upper layers. There was no evidence that the temperature was different between 0 and 6 m. The water temperature increased 5°C from day 0 to day 6. At the end of May, there is a seasonal change from spring to summer. The temperature of seawater in Xiamen Bay is 15°C higher than that in Saanich Inlet at the same time of the year.

Irradiance measured at 1100 h (Beijing time) in the bag and in the sea is shown in Fig. 6. In subtropical areas, the incidence angle of the sun is almost vertical at noon in the middle of June. The experiment was carried out close to this period. It was observed that sunlight penetrated from the surface to the bottom of the

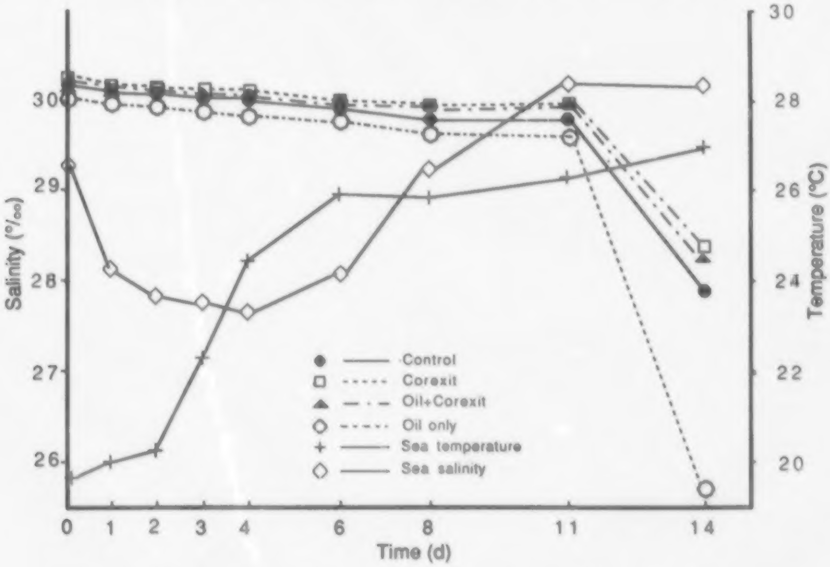

Fig. 5. Salinity in the field enclosure and in the sea, and temperature in the sea.

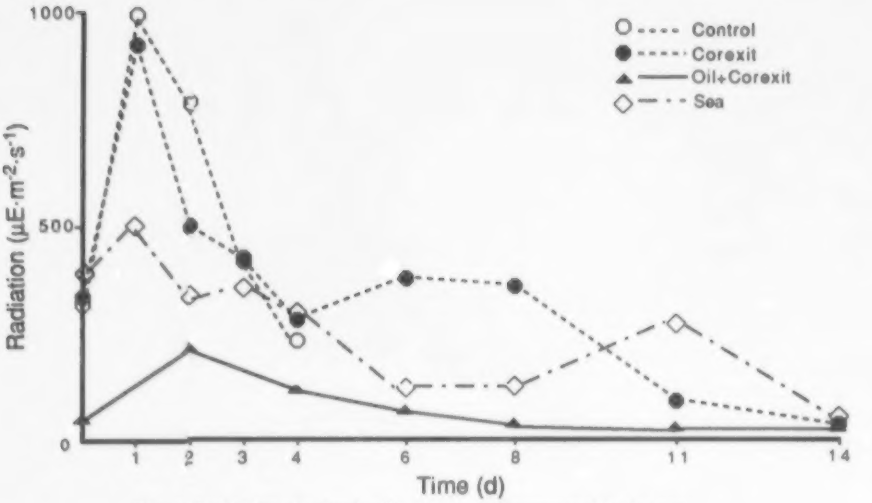

Fig. 6. Radiation at a depth of 2.5 m in enclosures and in the sea.

enclosure at noon. From day 6 to day 8, irradiance in the sea was quite low as a phytoplankton bloom occurred during this period. On day 14, the weather was cloudy, causing a reduction in radiation in the bags and in the sea. These results indicated that the light intensity in the seawater was controlled by the weather, the phytoplankton density, the bag material, and the flotation device, which could reduce sunlight at high latitudes for most of the day. Even so, radiation in the bags was generally higher than that in the sea, probably because of the reflection of the white plastic wall of the bag.

Nutrient limitation

The results of a study on nutrient limitation in Xiamen Bay from 1984 to 1986 show that both phosphorus and nitrogen limited phytoplankton production. However, phosphate was the primary limiting nutrient during the experimental period both in the bags and in the sea (Figs 7 and 8). Phosphate was depleted several days before nitrate, even though the ratios of nutrients added to the bags in the 1985 and 1986 experiments were N:Si:P = 10:10:1 and N:Si:P = 30:20:1 respectively. The mechanism of phosphorus limitation in the enclosure and the sea must be studied further.

Primary production

The primary productivity index in the bags and in the sea is shown in Fig. 9. It shows that the turnover rate in the bags was lower than that in the sea. It is assumed that the physiological activity of phytoplankton in the bag would be different from that in the sea. A phytoplankton bloom during this seasons generally occurs over a period of 15–20 d.

Sedimentation rate of organic particulates

One of the disadvantages of marine enclosure experiments is that vertical mixing is reduced because the water is enclosed. Therefore, physical processes in the

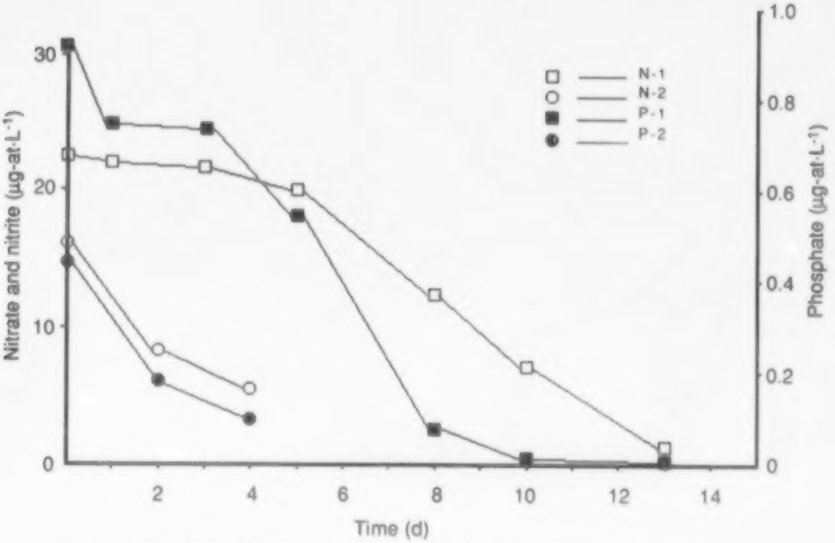

Fig. 7. Phosphate and nitrate–nitrite limitation in enclosures.

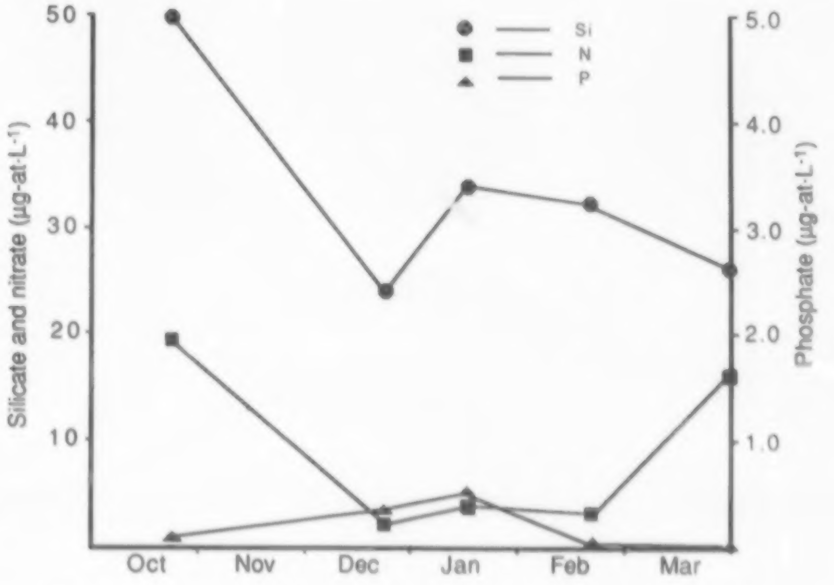

Fig. 8. Nutrient fluctuation at the experimental site in the eastern part of Xiamen Bay.

bag do not mimic those occurring in nature. This will affect the phytoplankton species succession structure. To test the enclosures, both in 1985 and 1986, some background investigations were carried out in Xiamen Bay before MEEE was

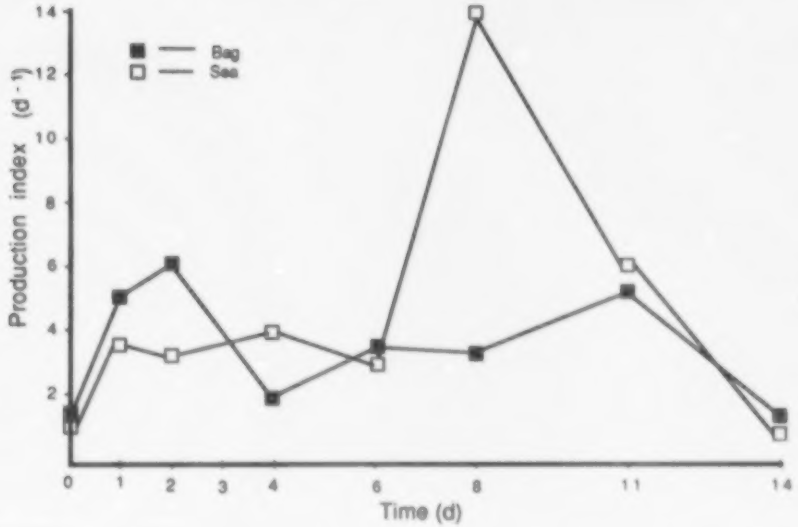

Fig. 9. Primary production in the control bag and in the sea.

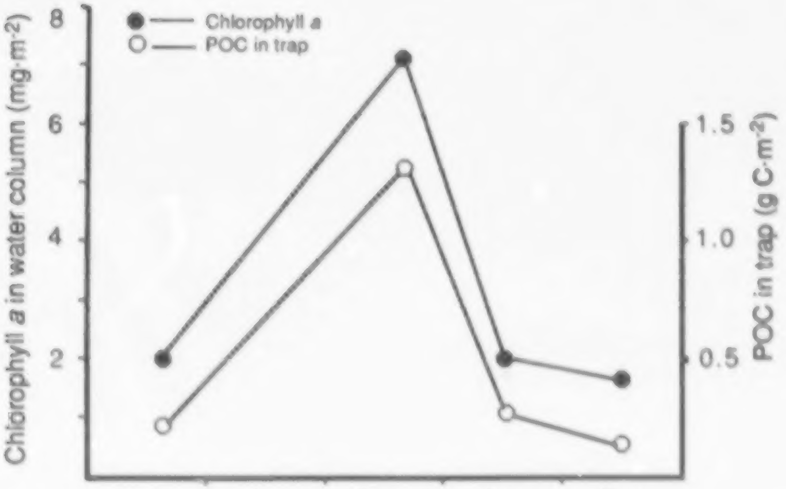

Fig. 10. Chlorophyll *a* and settled particulate organic carbon (POC) at the experimental site in the eastern part of Xiamen Bay.

conducted in the area. The ratio of POC of settled materials to total chlorophyll *a* in the whole water column was used as an index to estimate the possible difference in vertical mixing regime (Fig. 10). The sediment trap was positioned at 6 m, above which the layer was regarded as the euphotic zone where a phytoplankton bloom would occur.

The sedimentation rate in MEEE was compared with that in Xiamen Bay and in Saanich Inlet, Canada, and with that in SEAFLUX enclosures in 1983 and 1984. Results indicate that the data ranged from 2 to 100 mg d^{-1} (Table 2).

Sediment traps have been employed to trap sinking materials in the sea for some time (Gardner 1977). Debates have been raised about their use. From Table 2, it might be concluded that vertical mixing did affect the sinking rate of organic materials. Vertical mixing inside a trap is almost nil, especially when a grid is installed at the mouth of the trap to reduce mixing inside the trap. POC/chlorophyll *a* covered a wide range (about 10–100 d^{-1}) for sediment traps in Saanich Inlet and in Xiamen Bay. For SEAFLUX experiments in Saanich Inlet, Canada, with a local current velocity of 5 $cm \cdot s^{-1}$, and tank experiments (MEEE-85) in Xiamen, with turbulence occurring as circulation of water, the ratio was 3–5 d^{-1}. For field MEEEs in Xiamen in 1986, where the local current reaches more than 50 $cm \cdot s^{-1}$ over a certain period twice a day, causing good mixing in the enclosed water column, the ratio of POC to chlorophyll *a* was 2.1 d^{-1}. It would be interesting to compare ratios in other enclosures, in different areas and under different conditions. The results might reveal useful information for improving the MEEE design in the future and for comparing different types of sediment traps.

Pollution experiment results

Most of the enclosure experiments began by investigating pollution. The effects on the plankton ecosystem in our experiments are discussed here.

Changes in the concentration of chlorophyll *a* in the heavy metal experiment (Fig. 11a) show that the low concentration of metals did not significantly affect the phytoplankton community, but the high concentration of metals suppressed phytoplankton growth for a few days before it recovered in a short period. It was observed that larvacea (*Oikopleura* sp.) was very sensitive to heavy metals even at a level of a few parts per billion (Fig. 11b). *Oikopleura* was seriously suppressed by both low and high concentrations of metals, and did not recover even after 3 weeks.

The results of the crude oil and Corexit 9527 field MEEEs conducted in 1986 are shown in Fig. 11a–c. Centric diatoms were inhibited by crude oil pollution

Table 2. Comparison of sedimentation rates.

Experiment	POC settled ($mg\ C \cdot m^{-2} \cdot d^{-1}$)	Chlorophyll *a* (mg/m^2)	POC/chlorophyll *a* (d^{-1})
Xiamen background (trap)[a]	753	25.6	48±12
Saanich Inlet flux (trap)[b]	229	27.4	20±16
SEAFLUX-83-IOS	224	40.0	5.1
SEAFLUX-84-IOS	2 870	765	3.4
MEEE-85-TIO (in-tank)	81.2	20.0	4.0
MEEE-86-TIO (in situ)	75.1	36.6	2.1

[a] $n_1 = 5$, average of POC and chlorophyll *a* from October 1985 to May 1986.

[b] $n_2 = 16$, average of POC and chlorophyll *a* from October 1982 to November 1983.

(Fig. 11a), whereas there was no evidence to indicate that the chemical dispersant affected phytoplankton growth. The same result was observed for primary production (Fig. 11b). It is concluded that the plankton ecosystem was suppressed by the mixture of crude oil and chemical dispersant.

Crude oil stimulated the growth of marine bacteria (Fig. 12) compared with the

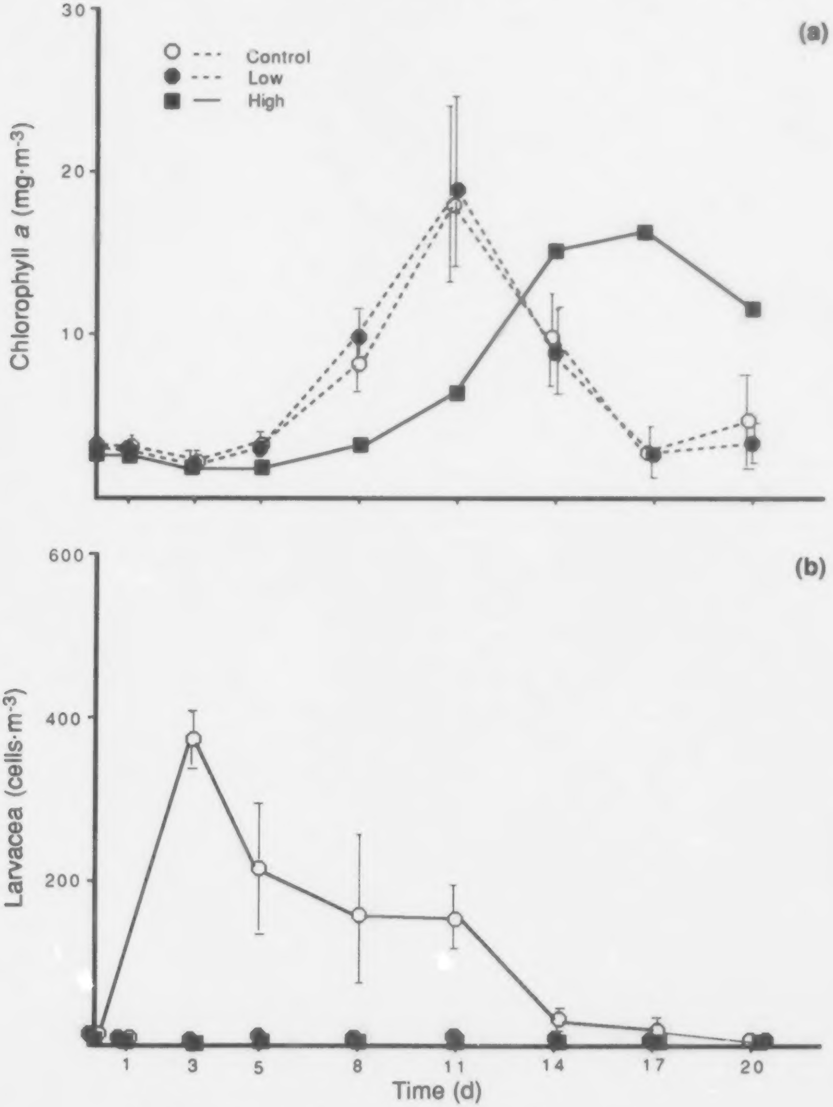

Fig. 11. (a) Chlorophyll *a* in heavy metal pollution experiments.
(b) Larvacea (*Oikopleura* sp.) inhibition by heavy metals.

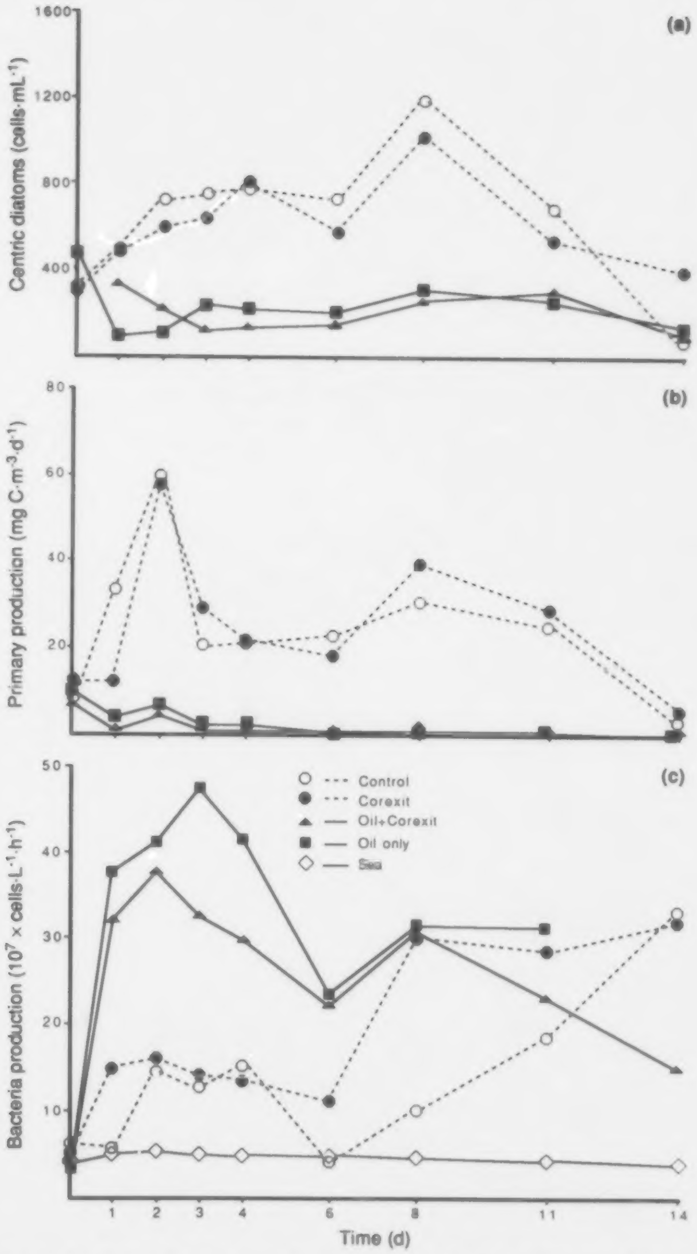

Fig. 12. (a) Centric diatoms in crude oil and Corexit 9527 pollution experiment.
(b) Primary production in crude oil and Corexit 9527 pollution experiment.
(c) Bacterial production dynamics in crude oil and Corexit 9527 pollution experiment.

control and the Corexit 9527 polluted enclosure. Bacterial production in the sea during this period was very stable compared with the control bag.

Improvement and development

The Xiamen MEEE is a modification of the SEAFLUX (CEPEX) system used in Saanich Inlet, Canada, but the environmental parameters at the two sites are quite different:

- The latitude of the SEAFLUX site is 49° N (temperate), whereas the Xiamen site is 24° N (subtropical), resulting in significant differences in irradiance and seawater temperature.
- Saanich Inlet is deep, calm, and has few local currents, whereas Xiamen Bay is relatively shallow, well-mixed, and has strong local currents.
- The plankton and ecological processes differ between the two sites, i.e., in nutrient limitation, density of plankton, and sizes of organisms.

After 4 years of cooperation, seven experiments have been conducted in China and Canada. It has been proven that the catamaran is an excellent flotation device for Xiamen Bay field enclosures. Bags have been launched at wind strengths of 5 Beaufort with 0.6-m waves, and the facility has withstood a large storm (7–8 Beaufort) with 1-m waves. Thus, the Xiamen MEEE has been set up successfully. Two types of enclosure facilities are very effective and have been used in studying plankton and ecosystem processes and the effect of pollution on them. However, many improvements are necessary for future experiments. The main improvements required are fivefold. First, for in-tank enclosures, a compromise must be reached between sunlight penetration and water temperature. Second, for field enclosures, the plastic bags and suspension system must be reinforced to withstand local currents, especially if experiments are to be carried out in the fall when local currents are strongest. Third, replicate and duplicate experiments are planned to estimate variations in the MEEE design and launching, sampling, and analytical methods. Fourth, background investigations will be continued in Xiamen Bay to compare field data with experimental data, both in the enclosure and in the laboratory. A system to study marine ecosystems and the material and energy fluxes in the ecosystem is planned that will employ three: microcosm in laboratory, enclosure (mesocosm), and field investigations. Fifth, an ecological model will be set up to describe carbon and energy flow both in enclosures and in Xiamen Bay.

Acknowledgments

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Davies, J.M., Gamble, J.C. 1979. Experiments with large enclosed ecosystems. Philosophical Transactions of the Royal Society of London (Series B, Biological Sciences), 286, 523–544.

Gardner, W.D. 1977. Fluxes, dynamics and chemistry of particulates in the ocean. Massachusetts Institute of Technology, Cambridge, MA, USA. PhD thesis, 405 pp.

Grice, G.D., Reeve, M.R. 1982. Introduction and description of experimental ecosystems. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 1–9.

Menzel, D.W., Case, J. 1977. Concept and designs: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 1–7.

Parsons, T.R., Maita, Y., Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, NY, USA. 173 pp.

Pilson, M. 1978. An adventure in studying marine ecosystems. Maritime, 22, 12–15.

Application of Different Types of Marine Experimental Enclosures to Study the Pathways and Fate of Chemical Pollutants

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Marine experimental enclosures systems have been developed at the Institute of Ocean Sciences to study the pathways and fate of chemical pollutants. These include the benthic boundary enclosure, with its wall attached to the seafloor; sediment seawater enclosure, with a sediment pan attached to the bottom; the portable multiple-enclosure system, in which a catamaran barge supports up to six fibreglass cylinders suspended from the barge so that seawater and materials collected at the site can be used for in situ studies. The application of these systems is reviewed with reference to marine studies of metal behaviour in seawater, metal release rates from contaminated sediments, the behaviour of mine tailings in seawater, and oil mixtures with dispersants, pentachlorophenol, and polychlorinated biphenyls (PCBs). The validity of observations of chemical behaviour in enclosed systems compared with that in natural systems is discussed.

The CEPEX (Controlled Ecosystem Pollution Enclosure Experiment) program sponsored by the United States National Science Foundation from 1973 to 1980, used mesoscale enclosures of seawater to study the biological effects of stress, natural or pollutant mediated, on marine ecosystems of bacteria, phytoplankton, zooplankton, and fish in the larval–juvenile stages. The enclosure systems, as described by Menzel and Case (1977) and Takahashi and Whitney (1977), were built for the purpose of capturing natural planktonic populations in their natural seawater environment to simulate a pelagic system.

The post-CEPEX enclosure programs, sponsored by the Institute of Ocean Sciences, were set up with a change in emphasis to pathways and fate of pollutants in the marine environment, particularly with reference to chemical fluxes. New developments in enclosure hardware were intended to couple pelagic and benthic processes to simulate situations where pollutants were exchanged between bottom water and polluted sediment. The enclosure systems were also streamlined for efficiency of operation, portability, and versatility as applied to field pollutant studies, such as ocean dumping, mine tailings disposal, and anoxicity.

This paper summarizes these new hardware developments and discusses their application to pathway studies and the biological effects observed during cooperative work between Canada and China under the bilateral Marine Ecosystem Enclosure Experiment (MEEE) program, sponsored by the International Development Research Centre (IDRC), and also during an experiment conducted with Japan under the cooperative program between Nagoya University, Tsukuba University, the University of Tokyo, and the Geological Survey of Japan.

Controlled ecosystem enclosures

The SEAFLUXES program at the Institute of Ocean Sciences improved the hardware originally described by Menzel and Case (1977) and Takahashi and Whitney (1977). The Controlled Ecosystem Enclosures (CEEs) were filled with seawater by scuba divers who compressed the bag to the bottom, usually at 22 m depth; oriented the bag mouth horizontally; and then swam back to the surface holding the bag mouth and connected it to the support float.

The CEPEX system used in 1977 experienced problems with working conditions. Recent improvements have enhanced durability, safety, and handling in rough weather. A rugged hexagonal surface float supported a woven polyethylene bag 2.5 m in diameter by 16 m deep suspended in water (Fig. 1). The conical bottom end of the bag was connected to a 38-mm diameter Tygon hose used to remove sedimented material by pumping. Six "shroud" lines suspended from the float with 25-kg weights at the end, dampened motion of the enclosure caused by mild ocean currents, generally <0.5 km·h^{-1} in Saanich Inlet, British Columbia. Elastic cords were used to attach the bag to the float, with the mouth of the bag about 30 cm above the sea surface, so that the float and the bag could move with some flexibility during rough weather.

Over the past 14 years, CEEs have been used to simulate various ecological conditions. These included artificial mixing (Eppley et al. 1978), elevated nutrient levels (Parsons et al. 1977), and altered carnivorous zooplankton populations (Reeve and Walter 1976). A typical CEE experiment simulated events during a spring bloom by adding a pulse of nutrients to stimulate phytoplankton growth, which subsequently resulted in nitrate depletion, a diatom bloom, and an increase in zooplankton, mainly copepods. In turn, the copepods were grazed by large animals, such as ctenophores and medusae (Fig. 2a). However, anomalous situations could create deviations from this typical pattern. For example, a high initial copepod population of over 10 000 m^{-3} (Fig. 2b) kept algal growth down in an uncontaminated CEE, whereas in a companion CEE, diatoms bloomed as a result of the addition of 8 µg·kg^{-1} of copper, which suppressed the copepod population (Whitney et al. 1981).

Sediment seawater enclosures

This system enclosed natural bodies of seawater and an undisturbed seabed in seawater. It was designed by the Ocean Chemistry Division at the Institute of

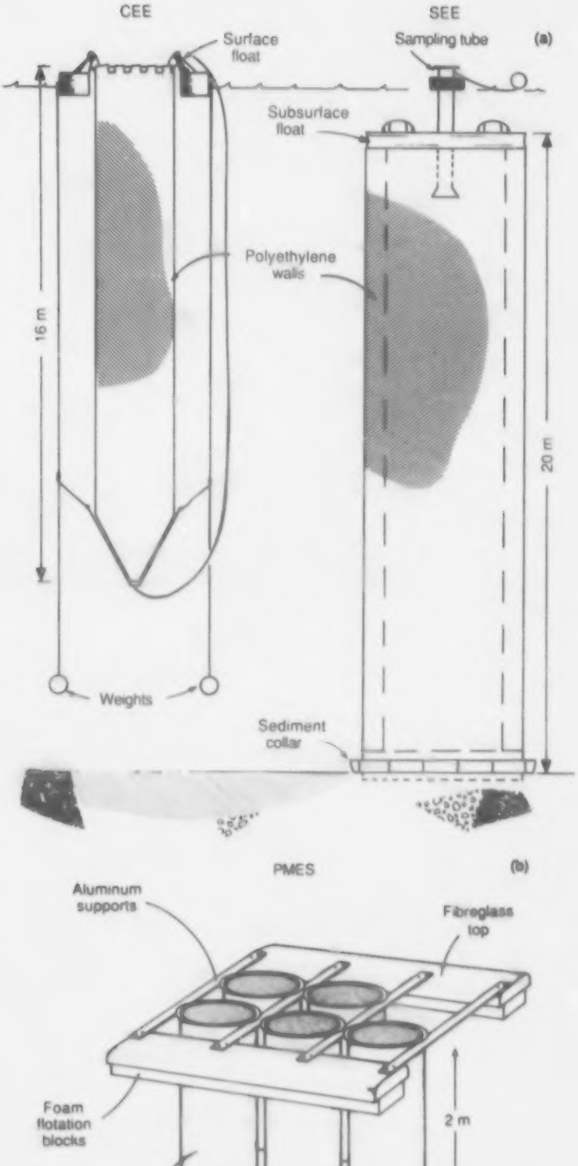
CEE
SEE
Surface float
Sampling tube
(a)
Subsurface float
Polyethylene walls
16 m
20 m
Weights
Sediment collar
PMES
(b)
Aluminum supports
Fibreglass top
Foam flotation blocks
2 m
Fibreglass tanks
1 m

Fig. 1. (a) Controlled ecosystem enclosure (CEE) redesigned from the quarter-scale CEPEX enclosures. Sediment seawater enclosure (SSE) anchored to the sediment by a steel and concrete collar, and kept taut by a buoyant top. (b) Portable multiple-enclosure system (PMES) consisting of a lightweight catamaran barge and up to six fibreglass tanks.

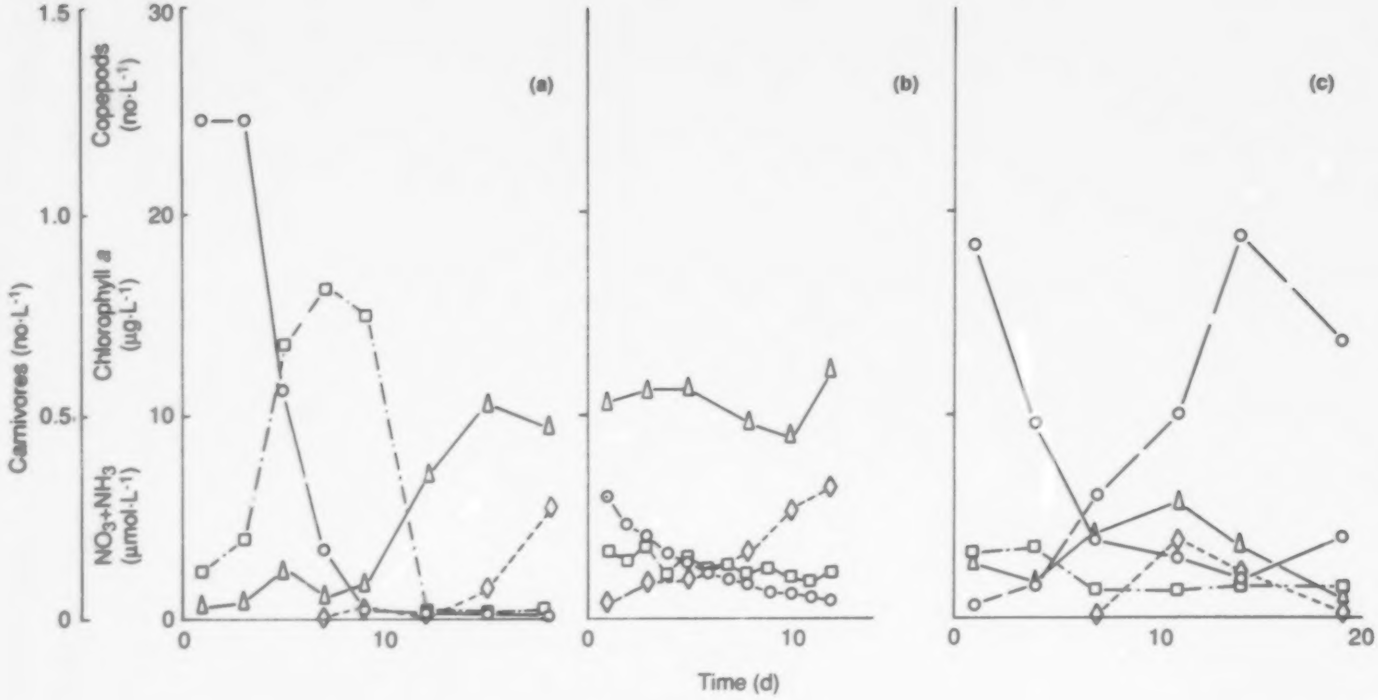

Fig. 2. (a) Typical series of events in a CEE as energy is transferred from phytoplankton to herbivores and then to carnivores. (b) Events in a CEE that had a high initial copepod population: no phytoplankton bloom occurs with the higher grazing rate. (c) Biological events under low light in a SSE.
Legend: NO_3, ○——○; NH_4, ○ - - - ○ (2c only); chlorophyll *a* (□) ; copepods (△); and carnivores, ctenophores, and medusae (◇).

Ocean Sciences for use in a joint Canada–Japan enclosure experiment on copper effects (Seki et al. 1983).

The system (Fig. 1a) had a woven polyethylene canopy, suspended in the water by attaching it to a subsurface float with 2 000 kg of buoyancy, to enclose a water column 5 m in diameter and 20 m high. The bottom of the canopy was anchored by a round steel collar weighing 4 000 kg in water and penetrating about 20 cm into the silty, sandy bottom. Nylon ropes were positioned between the top of the canopy and the collar to support the plastic enclosure.

Because the canopy top had to be below the low tide level in waters with a 3-m tidal range, sampling was accomplished from the surface with a capped polyvinyl chloride (PVC) sampling tube attached to the top of the canopy. This tube could be raised to the surface by buoyancy provided by compressed air injected into the tube through a hose connected to the surface float. The cap was then removed and equipment up to 30 cm in diameter could be lowered into the Sediment Seawater Enclosure (SSE). Seawater sampling could be carried out by peristaltic pumping through a polyethylene tube inserted into the sampling tube.

The enclosure created biological and physical characteristics not typical in the natural environment. Biological problems were encountered due to reduced light, blocked by the solid top and translucent walls of the SSE, particularly in the central part of the enclosure. A 100-fold decrease in light penetration occurred inside the SSE relative to that in the natural environment. Slower algal growth resulted in the

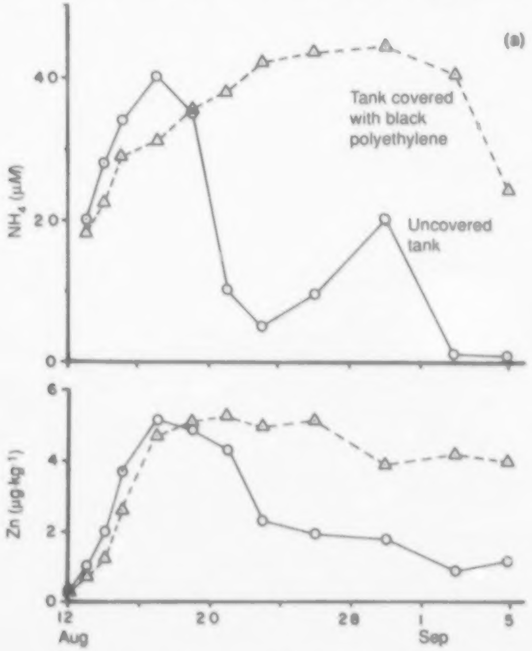

Fig. 3. (a) Changes in NH_4 and dissolved Zn concentrations in tanks containing a bed of sediments from False Creek.

SSE environment compared with previous work involving the more transparent CEEs. In the SSE, this slow buildup of algal biomass allowed enough time for herbivorous copepods to graze efficiently and for carnivores to develop fully.

The nutrient NO_3 utilized during the 1st week by algal growth was released back into the water as NH_4 due to excretion by herbivores, leading to extremely high NH_4 levels (Fig. 2c). This confirmed comments by Parsons et al. (1986) on the inverse relationship between light levels and amounts of grazing by zooplankton in an enclosure.

SSEs were severely affected by changes in the density of seawater inside and outside the enclosure. During one test, the inflow of denser water into the vicinity of the SSE percolated through the sediment, causing the lighter water in the enclosure to seep through the seams at the top, as indicated by the upward movement of dye injected into the water layer a few metres above the seafloor inside the SSE. In another test, when outside water became less dense than the water in the SSE, it created a bulge at the bottom of the SSE and the middle and top parts of the SSE collapsed. The changing shape of the SSE during the experiment severely limited its use to waters with a very stable density structure over the course of the enclosure study.

Portable multiple-enclosure system

The Portable Multiple-Enclosure System (PMES) consists of a catamaran barge, which can be assembled or taken apart easily, with 2–6 fibreglass tanks 1 m in diameter and 2 m deep suspended from the barge (Fig. 1b). A two-tank version was

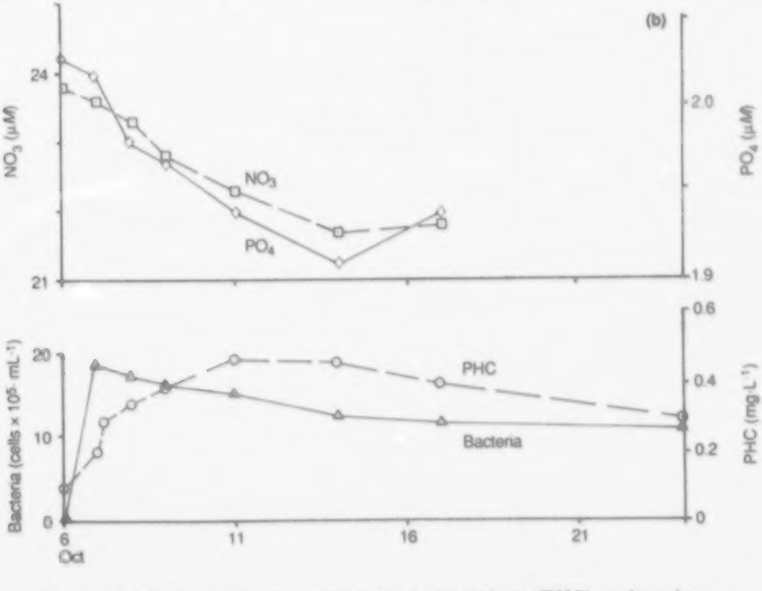

Fig. 3. (b) Changes in bacteria, particulate hydrocarbons (PHC), and nutrients in a PMES tank covered with black polyethylene.

used in a remote fjord for a mine tailings study, in which tailings-contaminated sediments, dredged on site from the seafloor, were added to seawater pumped from the 50-m depth (level of the tailings outfall) to study metal-release rates from the tailings into the fjord water (Zhan et al., this volume). A six-tank version was used in a metal-release study involving contaminated sediments from False Creek, British Columbia (Wong and Stukas, this volume).

This experimental system was useful in chemical and biological studies involving only lower trophic levels of bacteria and algae, which do not require a large volume enclosure to support this simple ecosystem. It also provided flexibility in creating experimental conditions, such as light and dark environments and oxic and anoxic regimes.

Figure 3a illustrates the coupling of nutrient and metal interactions in light and dark environments in an experiment using contaminated sediment from False Creek to simulate a seafloor on the bottom of fibreglass tanks. In the dark tank without sunlight, levels of both NH_4^+ and Zn increased rapidly initially and remained high for long periods afterward because of the lack of biological removal of these chemicals. In the tank with full sunlight, high productivity resulted in the removal of NH_4^+ and Zn from the seawater. Similar nutrient and metal coupling was observed in an open-ocean study (Bruland 1980).

Another example of the applicability of this system to an ecosystem study at the bacterial level is illustrated in Fig. 3b, in which bacterial degradation of a Chinese crude oil was investigated using the PMES. Decreases in nutrients and particulate hydrocarbons were related to increases in bacterial numbers; whereas for the control, nutrients and bacterial numbers remained unchanged in the absence of oil. Within 1 week after the oil addition, a decrease in oil, carbon, nitrogen (as $NO_3^- + NO_2^-$), and phosphate occurred at a ratio of 97:17:1 for C:N:P. A reduction of 11 $\mu mol \cdot L^{-1}$ of carbon in the particulate oil was coupled with a corresponding

Table 1. Half-life of pollutants in enclosures.

Materials	Reference	Half-life (d)	Conditions
Glucose, 4 ppm	Seki et al. (1983)	0.5	Remineralization
Crude oil, 20 ppm	Wong et al. (1984)	2–6	Sedimentation with diatoms
Crude oil, 0.5 ppm	Unpublished data	12	Remineralization
PCP, 10 ppb	Yunker (1981)	7.9	Photooxidation
PCP, 100 ppb	Yunker (1981)	8.5	Photooxidation
PCBs, 28 ppb	Iseki et al. (1982)	4.5	High productivity
		20	Low productivity
Cu, 50 ppb	Topping and Windom (1977)	31	Low productivity
Cu, 8 ppb	Whitney et al. (1981)	31	Low productivity
Hg, 0.1 ppb	Lu et al. (1986)	4.4	Diatom bloom
		30	Low productivity
Hg, 5 ppb	Wallace et al. (1982)	32	Low productivity
		7.6	High productivity
Cd, 1.3 ppb	Kremling et al. (1979)	40	High and low productivity
Pb, mine tailings	Wong et al. (1986)	9	High productivity
		20	Low productivity

increase of about 13 µmol·L^{-1} in bacterial biomass. Because bacterial growth also required substantial respiration of the utilized carbon, dissolved organic carbon must also be used as a carbon source.

Residence time of pollutants in enclosed water bodies

A summary of the half-life of organic and inorganic pollutants in enclosed seawater bodies used for marine ecosystem enclosure experiments carried out at the Institute of Ocean Sciences is presented in Table 1. The half-lives of metals used in the Institute of Ocean Sciences studies generally agreed with those from the enclosure work of Santschi et al. (1983) with emphasis on the interaction between metals in seawater and resuspended sediment. The Institute of Ocean Sciences studies focused mainly on the interaction of pollutants with biological uptake and mineralization associated with plankton blooms. This has relevance in the coastal waters of Western Canada where deep fjords receive pollutant discharges that would be

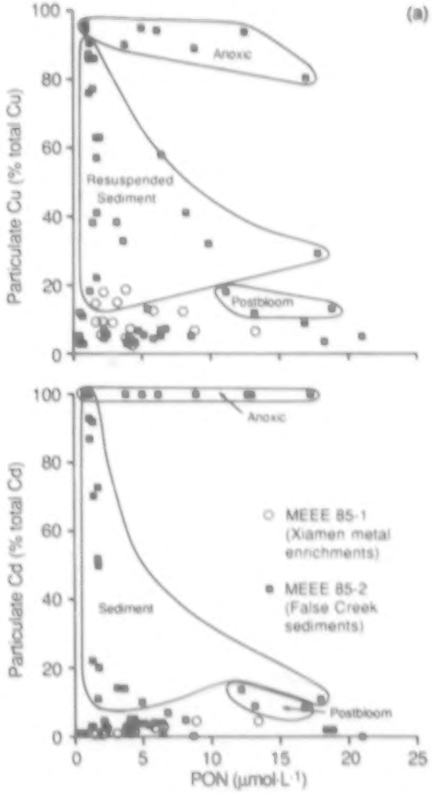

Fig. 4. (a) Percentages of total Cu and Cd bound to particles versus particulate organic nitrogen (PON). Data obtained from several environments inside enclosures, including anoxic waters, high suspended sediment load, and high to low biological activity.

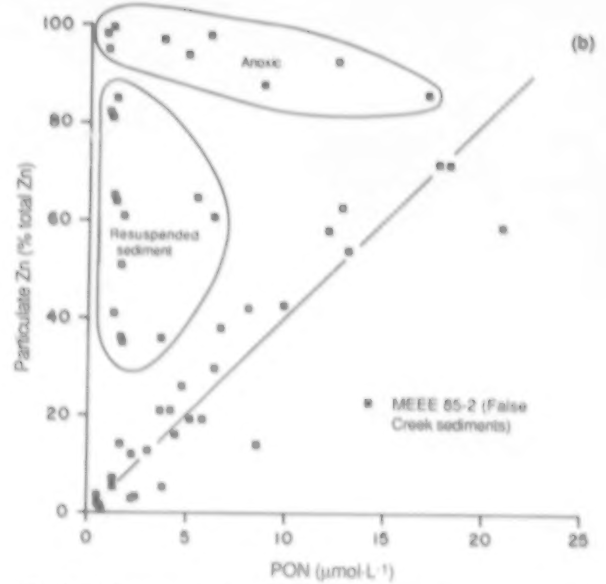

Fig. 4. (b) Percentage of total Zn bound to particles versus PON under the same circumstances as in (a).

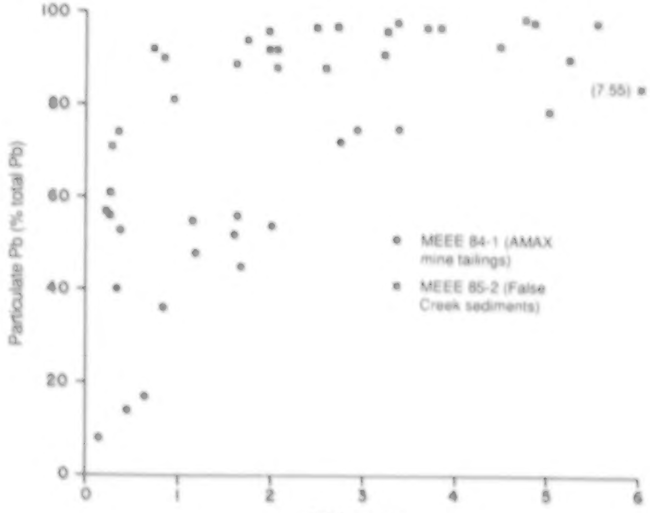

Fig. 5. Percentage of total Pb bound to particles versus suspended particulate material (SPM).

modified more by biological events in the euphotic zone than by resuspension events dominant in shallow bays.

Phytoplankton blooms shortened the half-lives of crude oil, polychlorinated biphenyls (PCBs), Hg, and Pb in enclosures by a factor of four to eight compared with those in the control without nutrient-mediated phytoplankton blooms. Those metals, such as Cd and Cu, with a low affinity for particles, had long half-lives. The half-lives were affected by a variety of pathways. For example, the shortened half-life of pentachlorophenol (PCP) was due to photooxidation, whereas those for crude oil and glucose were due to bacterial remineralization.

The particulate fractions of the metals were affected by the chemical states and condition of the sedimentation environment. Figure 4 shows the percentage of the particulate form of Cu, Cd, and Zn for MEEE studies on seawater interactions with contaminated sediments in fibreglass tank systems. The plots show the percentage of particulate metals against particulate organic nitrogen in seawater as an indicator of biological activity in the enclosure. Anoxicity would turn almost 100% of Cu, Cd, and Zn into the particulate form. Because of inorganic complexation in seawater (Nurenberg and Valenta 1983; Zuehlke and Kester 1983), Cd and Cu in seawater showed a low affinity for particles, resulting in a very small increase in the particulate metals associated with the particulate phase in seawater or with the settling material collected immediately after a diatom bloom (Fig. 4a).

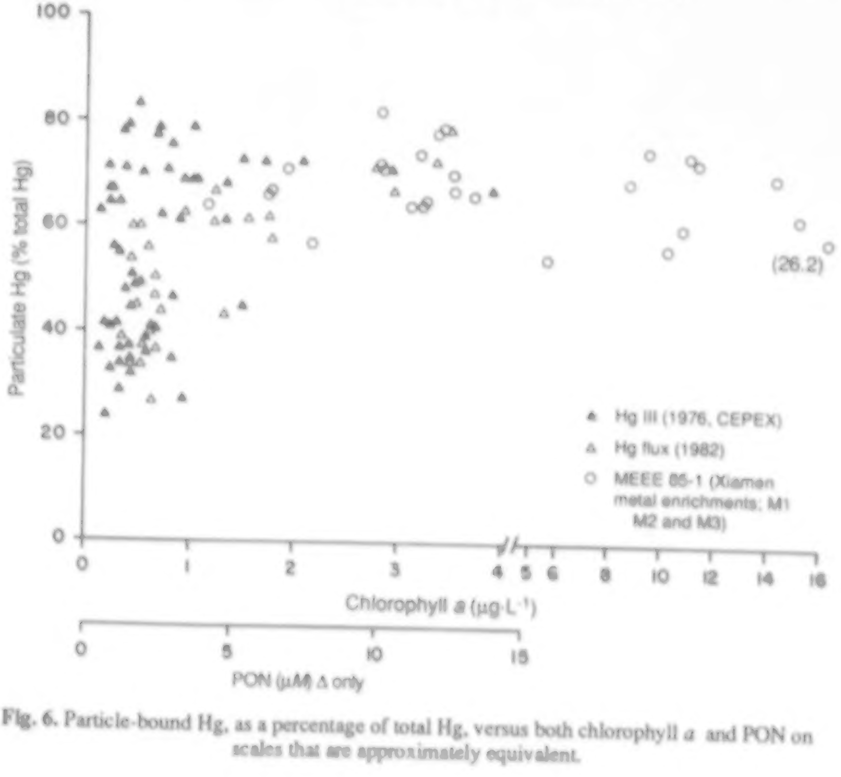

Fig. 6. Particle-bound Hg, as a percentage of total Hg, versus both chlorophyll *a* and PON on scales that are approximately equivalent.

Although all of these metals are considered to be biologically active, Zn exhibited far more activity with the biological state of the enclosure, as indicated by the linear correlation of the percentage of particulate Zn with particulate organic nitrogen illustrated in Fig. 4b. In contrast, metals such as Pb and Hg showed a high particle affinity. Figure 5 shows that particulate Pb would be mainly associated with inorganic suspended matter in seawater based on experiments using mine tailings and contaminated near-shore sediment. Figure 6 shows that particulate Hg, the dominant component of total Hg in the enclosed experiments, would be mainly associated with organic particles. Significant biological removal was observed in CEPEX Hg experiments (Wallace et al. 1982), in which the half-life of Hg was shortened to 8 d compared with 32 d when algal growth was suppressed.

The enclosure approach yields a better understanding of the pathways and fate of pollutants, both organic and inorganic, in the marine environment, and it is useful for the formulation of a better strategy for ocean dumping and the protection of coastal resources. Capping contaminated sediment in ocean dumping is a good example (Wong and Stukas, this volume).

Bruland, K.W. 1980. Oceanographic distribution of cadmium, zinc, nickel and copper in the North Pacific. Earth and Planetary Science Letters, 47, 176–198.

Eppley, R.W., Koeller, P., Wallace, G.T. 1978. Stirring influences the phytoplankton species composition within enclosed columns of coastal seawater. Journal of Experimental Marine Biology and Ecology, 32, 219–239.

Iseki, K., Takahashi, M., Bauerfeind, E., and Wong, C.S. 1982. Effects of polychlorinated biphenyls (PCBs) on a marine plankton population and sedimentation in controlled ecosystem enclosures. Marine Ecology Progress Series, 5, 207–214.

Kremling, K., Puize, J., von Brockel, K., Wong, C.S. 1979. Studies on the pathways and effects of cadmium in controlled ecosystem enclosures. Marine Biology, 48, 1–10.

Lu, X., Johnson, W.K., Whitney, F.A., Wong, C.S., Wu, J. 1986. A study on flux, speciation and budget of mercury in controlled experimental ecosystems. Oceanologia et Limnologia Sinica, 17, 307–317.

Menzel, D.W., Case, J. 1977. Concept and design: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 1–7.

Nurenberg, H.W., Valenta, P. 1983. Potentialities and applications of voltametry in chemical speciation of trace metals in the sea. *In* Wong, C.S., Boyle, E., Bruland, K., Burton, J.S., Goldberg, E.D., ed., Trace metals in seawater. Plenum Publishing, New York, NY, USA.

Parsons, T.R., von Brockel, K., Koeller, P., Takahashi, M., Reeve, M.R., Holm-Hansen, O., 1977. The distribution of organic carbon in a marine planktonic food web following nutrient enrichment. Journal of Experimental Marine Biology and Ecology, 26, 235–247.

Parsons, T.R., Thompson, P., Wu, Y., Lalli, C.M., Hou, S., Xu, H. 1986. The effect of mine tailings on the production of plankton. Acta Oceanologica Sinica, 5, 417–423.

Reeve, M.R., Walter, M.A. 1976. A large-scale experiment on the growth and predation potential of ctenophore populations. *In* Mackie, G.O., ed., Coelenterate ecology and behavior. Plenum Publishing, New York, NY, USA.

Santschi, P.H., Adler, D.M., Amdurer, M. 1983. The fate of particles and particle-reactive trace metals in coastal waters: radioisotope studies in microcosms. *In* Wong, C.S., Boyle, E., Bruland, K., Burton, J.S., Goldberg, E.D., ed., Trace metals in sea water. Plenum Publishing, New York, NY, USA.

Seki, H., Whitney, F.A., Wong, C.S. 1983. Copper effect on dynamics of organic materials in marine controlled ecosystems. Archiv fuer Hydrobiologie, 96, 176–189.

Takahashi, M., Whitney, F. 1977. Temperature, salinity and light penetration structures: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 8–16.

Topping, G., Windom, H.L. 1977. Biological transport of copper at Loch Ewe and Saanich Inlet: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 135–141.

Wallace, G.T., Seibert, D.L., Holzknecht, S.M., Thomas, W.H. 1982. The biogeochemical fate and toxicity of mercury in controlled experimental ecosystems. Estuarine and Coastal Marine Science, 15, 151–182.

Whitney, F.A. ed. 1984. The effects and fate of chemically dispersed crude oil in a marine ecosystem enclosure — data report and methods. Institute of Ocean Sciences, Department of Fisheries and Oceans, Sidney, BC, Canada. Canada Data Report of Hydrography and Ocean Sciences 29, 77 pp.

Whitney, F.A., Philpott, C.L., Seki, H., Wong, C.S. 1981. The effects of copper and copper plus glucose on an enclosed marine ecosystem. Institute of Ocean Sciences, Department of Fisheries and Oceans, Sidney, BC, Canada. Pacific Marine Science Report 81-21, 69 pp.

Wong, C.S., Whitney, F.A., Cretney, W.J., McLaughlin, F., Wu, J., Fu, T. Zhuang, D. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527: Part I — Fate of a chemically dispersed crude oil. Marine Environmental Research, 13, 247–263.

Wong, C.S., Whitney, F.A., Johnson, W.K., Lu, X., Wu, J., Wu S., Li, J. 1986. The effect of mine tailings on levels of heavy metals in seawater. Acta Oceanologica Sinica, 5, 401–415.

Yunker, M.S., Whitney, F.A., Cretney, W.J., Wong, C.S. 1990. An experimental marine ecosystem study of the pelagic biogeochemistry of pentachlorophenol. Marine Environment Research, 30, 273–295.

Zuehlke, R.W., Kester, D.R. 1983. Copper speciation in marine waters. *In* Wong, C.S., Boyle, E., Bruland, K., Burton, J.S., Goldberg, E.D., ed., Trace metals in sea water. Plenum Publishing, New York, NY, USA.

Effect of Vertical Mixing on Ecosystem Dynamics in Large Mesocosms

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The effect of vertical mixing on ecosystem dynamics in a large mesocosm has been examined using an ecotrophic computer-simulation model accounting for turbulent diffusion within a Controlled Ecosystem Pollution Experiment (CEPEX)-type enclosure. Over a range of turbulent diffusivity in the thermocline from 0 to 1 $cm^2 \cdot s^{-1}$*, changes in the standing stock of plankton were followed for a simulated period of 60 d. Assuming initial conditions to be similar to those in large CEPEX enclosure experiments, the increased turbulent diffusion caused better phasing between primary, secondary, and tertiary producers, resulting in a higher standing stock of ctenophores in model simulations with the highest vertical diffusivity. Conversely, the highest initial standing stock (or "bloom") of phytoplankton occurred in simulations in which the vertical diffusivity was zero. These results have been compared with actual CEPEX experiments.*

Enclosure of large volumes of seawater in plastic bags should ideally reduce the horizontal movement of water while maintaining vertical movement. However, because horizontal and vertical movements of water are physically coupled, exclusion of horizontal motion damps out much of the vertical transport in the water column. At the same time, vertical motion may be affected by temperature differences in the water inside and outside the bags. These problems were studied by Steele et al. (1977) who estimated average coefficients of turbulent diffusivity within Controlled Ecosystem Pollution Experiment (CEPEX) enclosures (60–1 300 m^3) to be in the range of 0.05–0.26 $cm^2 \cdot s^{-1}$ in the thermocline. Using a simulation model, the authors were able to account for the approximate distribution of chlorophyll and nutrients found in a 60-m^3 CEPEX enclosure.

In another approach to the lack of vertical mixing within large plastic enclosures, Sonntag and Parsons (1979) attempted to increase vertical mixing experimentally through the sporadic release of bubbles at 15 m in a 1 300-m^3 CEPEX enclosure. The purpose of this experiment was to study the effect of increased mixing on the trophodynamics of a food chain from phytoplankton to young fish and ctenophores. Although the results of these experiments were extensive, the authors had to conclude that "bubbling of the bags with compressed air did not serve as an adequate simulator of upwelling." The reason for this was that bubbling created large convective cells with sporadic intense vertical circulation instead of a sustained gentle upwelling.

In the experiments reported here, an attempt has been made to combine the modeling approach of Steele et al. (1977) and the upwelling approach of Sonntag and Parsons (1979) by examining the effect of changes in turbulent diffusivity on an ecosystem trophodynamic model that includes all of the principal components measured in the CEPEX experiment conducted by the latter authors. [illegible] computer model was the mixed upper-layer ecosystem simulation (MULES) model (Parsons and Kessler 1986), which was extended into a two-layer system of mixed layer and thermocline in the manner discussed by Taylor et al. (1986).

Methods

The MULES model was applied to generate values of all of its state variables for each layer. A simplified flow design for the MULES system is illustrated in Fig. 1. The version employed differed from that of Parsons and Kessler (1986) in that autotrophic flagellates, heterotrophic flagellates, and microzooplankton are all included; the flagellates being grazed by the microzooplankton, which are, in turn, grazed by the macrozooplankton. Diatoms were grazed directly by the macrozooplankton. The flow design of the model as shown in Fig. 1 includes changes from the original model of Parsons and Kessler (1986), which are shaded in gray.

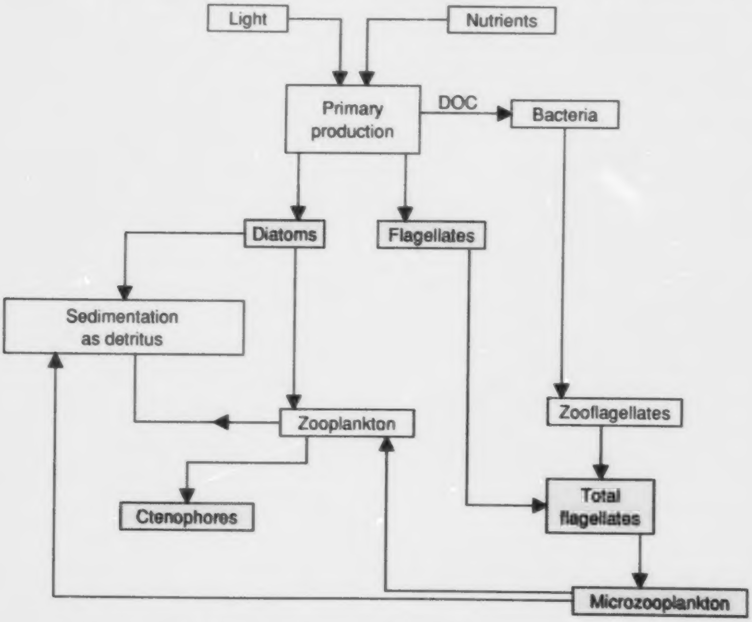
Light
Nutrients
Primary production
DOC
Bacteria
Diatoms
Flagellates
Sedimentation as detritus
Zooplankton
Zooflagellates
Ctenophores
Total flagellates
Microzooplankton

Fig. 1. Simplified diagram of the modified MULES model (Parsons and Kessler 1986). Shaded boxes are those that have been changed from the original model (DOC = dissolved organic carbon).

In the course of calculating primary production, the MULES model calculates a light profile down each layer. By applying the light intensity at the bottom of the mixed layer as the surface irradiance at the top of the thermocline, shading of phytoplankton in the thermocline by those in the mixed layer was included.

Following Taylor et al. (1986), rates of increase or decrease of any state variable X were described by two equations:

[1] $\mathrm{d}X_\mathrm{M}/\mathrm{d}t = M_\mathrm{u}(X_\mathrm{M}) - vX_\mathrm{M}/h_\mathrm{M} + k(X_\mathrm{T} - X_\mathrm{M})/h_\mathrm{M}$

[2] $\mathrm{d}X_\mathrm{T}/\mathrm{d}t = M_\mathrm{u}(X_\mathrm{T}) - v(X_\mathrm{T} - X_\mathrm{M})/h_\mathrm{T} + k(X_0 - X_\mathrm{T})/h_\mathrm{T} - k(X_\mathrm{T} - X_\mathrm{M})h_\mathrm{M}$

where X_u and X_T are the concentrations of X in the mixed layers and thermocline, $M_\mathrm{u}(X_\mathrm{M})$ and $M_\mathrm{u}(X_\mathrm{T})$ are the increases and decreases of X resulting from the MULES system, v is the sinking speed appropriate for X, k is a vertical mixing coefficient representing turbulent transfer between the layers, and h_M and h_T are the thicknesses of the two layers. X_0 is the concentration of X below the thermocline; this was assumed to be zero for all variables except the concentration of dissolved nitrate.

For the purpose of the experiments described here, the depth of mixing within a CEPEX enclosure was assumed to be 15 m, with a thermocline of 5 m thickness beneath this. The ambient temperatures in the mixed layer and thermocline were taken to be 15 and 10°C respectively. The vertical mixing coefficient has the dimensions of speed. Its value was estimated by requiring that the time taken to cross the thermocline, whose thickness is Z m, be the same as that taken by particles spreading within the thermocline under Fickian diffusion to reach a mean square displacement of Z, namely $Z^2/(2K)$. For typical thermocline values of the eddy diffusivity K (i.e., 0.1–1 $\mathrm{cm}^2\cdot\mathrm{s}^{-1}$), k has values of 0.01-0.1 $\mathrm{m}\cdot\mathrm{h}^{-1}$.

Results

Mixed layer starting values are listed in Table 1 for the three experiments in which mixing was varied from 0 to 0.01 to 0.1 $\mathrm{m}\cdot\mathrm{h}^{-1}$; all other values are the same as in Parsons and Kessler (1986). These values were held constant and only the intensity of mixing was changed between each of the 70-d simulations during which the plankton community was followed. The results are shown in Fig. 2 as changes in the biomass of phytoplankton (chlorophyll *a*), zooplankton, and ctenophores.

Table 1. Initial parameters used in the experiment.

Depth of mixing	15 m
Nitrate	15 μM
Chlorophyll *a*	1 $\mathrm{mg}\cdot\mathrm{m}^{-3}$
Zooplankton	3 $\mathrm{mg\ C}\cdot\mathrm{m}^{-3}$
Ctenophores	0.001 $\mathrm{mg\ C}\cdot\mathrm{m}^{-3}$
Surface radiation	1 000 $\mu\mathrm{E}\cdot\mathrm{m}^{-2}\cdot\mathrm{s}^{-1}$
Extinction coefficient	0.07 m^{-1}
Temperature	15°C
Nitrate below the mixed layer	20 μM

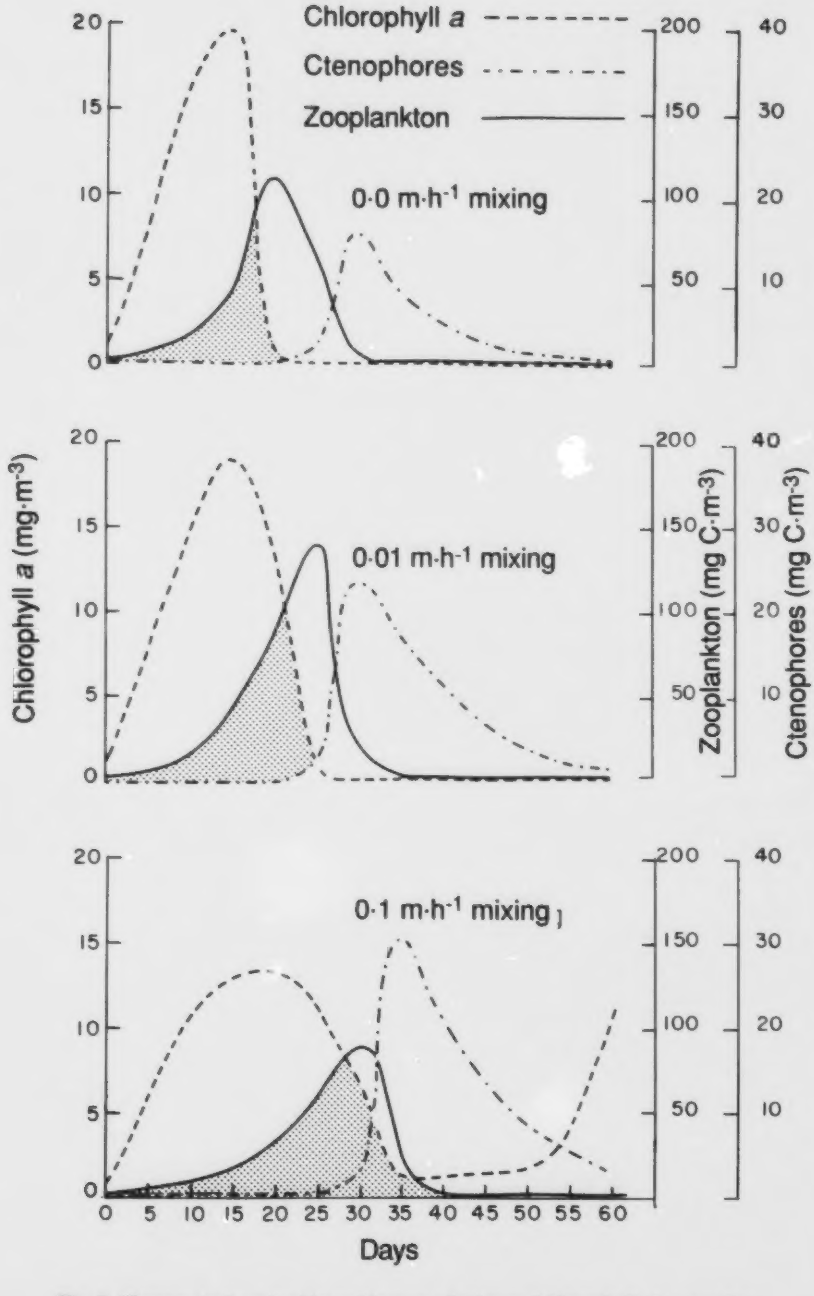

Fig. 2. Changes in the mixed layer biomass of primary (chlorophyll *a*), secondary (zooplankton), and tertiary (ctenophores) producers as result of different mixing rates.

The pattern of events generated by the model shows that the standing stock of tertiary producers (the ctenophores) is progressively increased by the degree of mixing. Although this may appear obvious, because more nitrate is supplied to the whole ecosystem by the greatly increased mixing, the mechanism by which the increased supply of nitrogen is transferred up the food chain is much less predictable. The peak zooplankton standing stock, for example, first increases in 0 to 0.01 $m \cdot h^{-1}$ mixing regimes (i.e., from about 100 to 125 mg $C \cdot m^{-3}$) and then decreases to about 75 mg $C \cdot m^{-3}$ in the most severely mixed water column. Similarly, the chlorophyll concentration shows a maximum of more than 20 mg chlorophyll $a \cdot m^{-3}$ in the absence of mixing, but reaches <15 mg chlorophyll $a \cdot m^{-3}$ in the water column mixed at 0.1 $m \cdot h^{-1}$.

These differences in the standing stock results at the first two trophic levels are not directly related to the standing stock of tertiary producers. What is related to the standing stock of tertiary producers is the extent of phasing between the various levels of production. This is illustrated in Fig. 2 by shading the phased portion of the primary and secondary standing stock curves. For 0 $m \cdot h^{-1}$ mixing, the phased portion of these two curves covers a period of 20 d, whereas for 0.01 and 0.1 $m \cdot h^{-1}$ mixing, the phased portions of the curves cover periods of 27 and 40 d, respectively.

What actually happens is that, under conditions of zero mixing, the phytoplankton use all available nitrate before the slower-growing zooplankton have a chance to graze much of the phytoplankton growth. The phytoplankton sink out and are then no longer available to the zooplankton. In the mixed-water columns, phytoplankton growth is sustained for a longer period by the mixing; zooplankton production is, consequently, better sustained; and more zooplankton are available for ctenophore grazing per unit of time. This results in a larger amount of the zooplankton being transferred to the ctenophore population. Thus, it is not so much a matter of increasing the nitrate supply as it is of sustaining the primary productivity and, thereby, the phasing of the whole system with the result of higher tertiary production.

It is important to note, however, that in the absence of vertical mixing phytoplankton and zooplankton can only be lost from the mixed layer by sinking (i.e., sedimenting out). When there is mixing between the mixed layer and the thermocline, nutrients can be brought into the mixed layer from below, and at the same time phytoplankton and zooplankton can be transported out of the mixed layer by turbulent mixing. These turbulent losses are in addition to those resulting from grazing. As ctenophores are strong swimmers, it was assumed in the model that they were not subject to loss by sinking or turbulent transport. Because of turbulent losses, phytoplankton and zooplankton each increase more slowly with increased mixing.

These results are a partial explanation for some of the data reported by Sonntag and Parsons (1979) who simulated upwelling in controlled ecosystems. In their data, there was no obvious sequence of events in the standing stock levels of primary and secondary producers with upwelling. In fact, the standing stocks of chlorophyll and zooplankton were often lower in the upwelled containers than in the control (no mixing). However, primary productivity in the two mixed-water columns was up to 50% greater than in the control and in one container in which ctenophore grew, the standing stock of ctenophores was about 100% higher than in the unmixed control. These results are consistent with data illustrated in Fig. 2.

Conclusions

The effect of mixing on controlled ecosystems is shown in Fig. 2 to result in a larger standing stock of tertiary producers (ctenophores). In contrast, the standing stocks of phytoplankton and zooplankton do not show a progressive increase with mixing; this is attributed to the degree of phasing between the trophic levels. When these results are compared with experimental results reported earlier, a similar pattern is observed with respect to changes in standing stocks of primary, secondary, and tertiary producers. The results emphasize the importance of "trophic phasing dynamics" in marine ecosystems as described in earlier publications regarding salmon survival (Parsons and Kessler 1987).

Parsons, T.R., Kessler, T.A. 1986. Computer model analysis of a pelagic ecosystem in estuarine waters. *In* Skreslet, S., ed., The role of freshwater outflow in coastal marine ecosystems. Springer-Verlag, New York, NY, USA. 161–181.

______ 1987. An ecosystem model of the assessment of plankton production in relation to the survival of young fish. Journal of Plankton Research, 9, 125–137.

Sonntag, N.C., Parsons, T.R. 1979. Mixing an enclosed, 1300 m^3 water column: effects on the planktonic food web. Journal of Plankton Research, 1, 85–102.

Steele, J.H., Farmer, D.H., Henderson, E.W. 1977. Circulation and temperature structure in large marine enclosures. Journal of the Fisheries Research Board of Canada, 34, 1 095–1 104.

Taylor, A.H., Harris, J.R.W., Aiken, J. 1986. The interaction of physical and biological processes in a model of the vertical distribution of phytoplankton under stratification. *In* Nihioul, J.C.J., ed., Marine interfaces ecodynamics. Elsevier, Amsterdam, Netherlands. 313–330.

Phosphate Limitation of Phytoplankton Growth in Coastal Estuarine Waters of China and Its Potential Interaction with Marine Pollutants

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Mesocosm experiments conducted in estuarrine coastal waters at Xiamen, People's Republic of China, indicate that phosphate is the nutrient that limits phytoplankton production. During these experiments, phytoplankton grew in marine enclosures and phosphate always reached undetectable levels several days before inorganic nitrogen ($NO_3^- + NO_2^- + NH_4^-$)*. This observation was supported by the N:P ratio in the water at the start of the experiment. The N:P ratio was nearly 80:1 (by atoms), indicating a fivefold excess of nitrogen, assuming that phytoplankters take up nitrogen and phosphorus in a N:P ratio of 16:1. Laboratory bioassay experiments with the diatom* Chaetoceros calcitrans *also confirmed that phosphorus was limiting to phytoplankton growth in Xiamen Bay. These estuarine coastal waters are unique because marine waters are usually nitrogen-limited. Several other estuaries along the coast of China also have N:P ratios ranging from 30:1 to greater than 80:1. These observations suggest that many of China's large rivers appear to act like giant nutrient pumps, delivering excess nitrogen, relative to phosphorus, to the coastal waters. The consequences of phosphorus limitation in relation to eutrophication and the interaction of phosphorus-limited phytoplankton with pollutants such as oil and heavy metals are discussed.*

Nitrogen is generally regarded as the limiting element for phytoplankton growth in the ocean (Ryther and Dunstan 1971; Goldman 1975). During recent mesocosm experiments conducted in estuarine coastal waters of the People's Republic of China, this general statement was found to be untrue. Experiments and bioassay results suggest that phosphorus is clearly limiting in the estuarine coastal waters of Xiamen Bay and may be limiting in several other estuarine areas along the coast.

Mesocosm experiments

Canada

In mesocosm experiments conducted at the Institute of Ocean Sciences in Saanich Inlet, British Columbia, and in Controlled Ecosystem Pollution Experiments (CEPEX) conducted in the same inlet (Grice and Reeve 1982), inorganic nitrogen was always exhausted in the mesocosms before inorganic phosphate (Harrison et al. 1986; Parsons et al. 1986). These observations along with the initial N:P ratio in the water (usually lesser than 16:1) provide evidence that nitrogen limits phytoplankton growth in Canadian coastal waters (Harrison et al. 1983).

China

The disappearance of nutrients in mesocosm experiments in China was very different from results obtained in Canada. During a sediment- and metal-addition experiment conducted at Xiamen in 1985 (Wu J. et al., this volume), with an initial N:P ratio of 32:1 in the seawater, phosphate went to undetectable concentrations 1 week before nitrate in the control mesocosm (Fig. 1). On day 10, when the phosphate concentration went to zero, there was still 15 μM nitrate left.

Further bioassay experiments were conducted to confirm that phosphorus was limiting to phytoplankton growth. Xiamen Bay water was collected in March and the diatom *Chaetoceros calcitrans* was used as the bioassay organism. The initial nitrate and phosphate concentrations were 17.0 and 0.21 μM, respectively, yielding a N:P ratio of 80:1 (by atoms). In one flask, only phosphate (0.8 μM) was added, giving a N:P ratio of 16:1. In another flask, only nitrate (122 μM) was added,

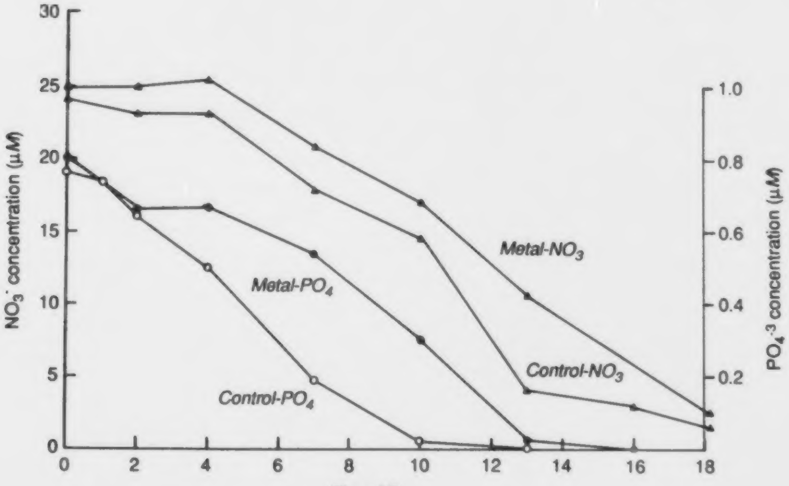

Fig. 1. Disappearance of nitrate and phosphate during a metal-addition experiment in mesocosms in Xiamen in 1985.

giving a N:P ratio of 656:1. In yet another flask, both N (122 μM) and P (0.8 μM) were added, giving a N:P ratio of 130:1. When N plus P and only P were added, fluorescence doubled compared with the control (no additions) (Fig. 2). When only N was added, fluorescence did not increase in over the control. Because only the addition of phosphate caused an increase in fluorescence, it was concluded that the water was phosphate-limited.

A plot of the seasonal variation in the N:P ratio in Xiamen Bay water and the Changjiang estuary (Yangtze River estuary near Shanghai) revealed that the ratio was high in the winter and spring (N:P = 30:1–40:1), decreased rapidly in late summer to less than 20:1, and then increased again in the fall (Fig. 3). It is important to note that all of these ratios are greater than 16:1, the Redfield ratio, except for one value recorded in July. The phosphate concentration decreased steadily from winter to spring, reaching a minimum in May (Fig. 3). In contrast, nitrate remained constant during the winter (Fig. 3). Reduced light due to the heavy suspended load in the water may affect nitrate uptake more than phosphate uptake during the winter.

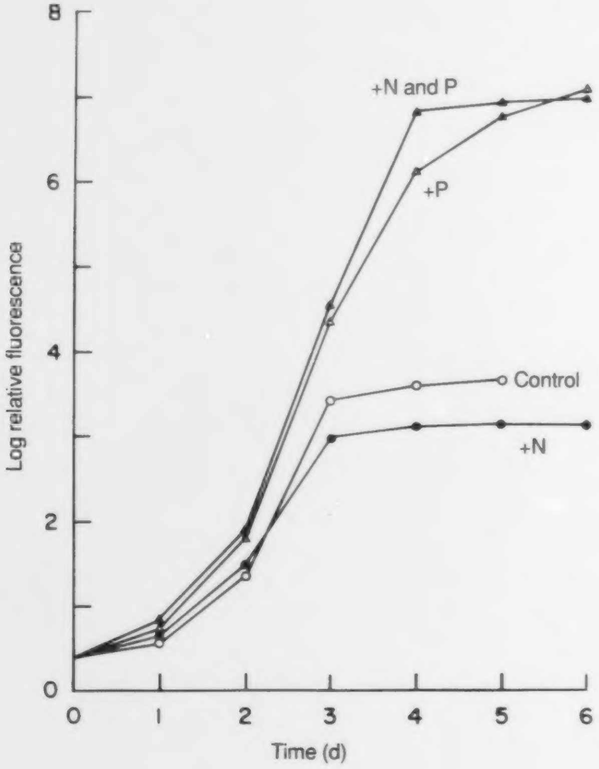

Fig. 2. Bioassay experiment in which water collected from Xiamen Bay in March 1986 was assayed for N or P limitation using a diatom, *Chaetoceros calcitrans*, as an assay organism. The control represents no nutrient additions, whereas +N = 122 μM and +P = 0.8 μM.

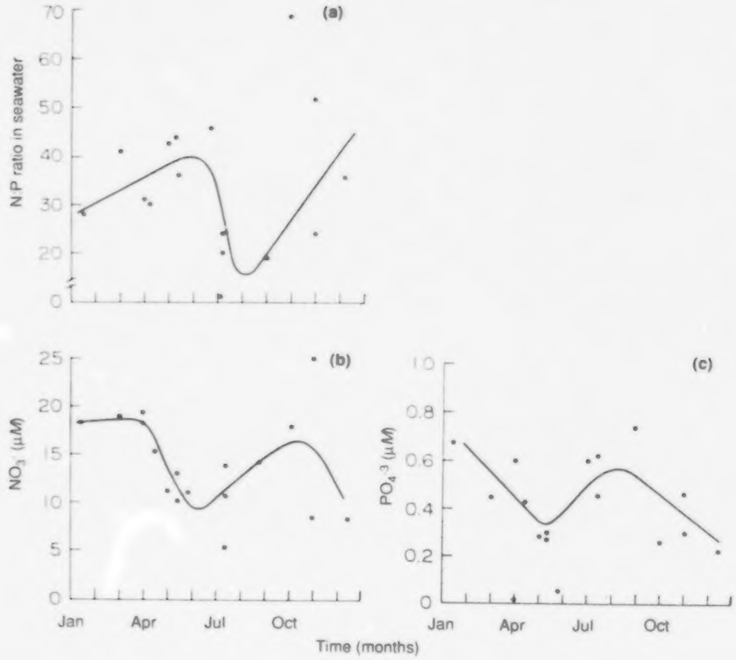

Fig. 3. **(a)** Seasonal variation in N:P ratios (by atoms) in seawater (nitrate + phosphate) from Xiamen Bay and Changjiang estuary; **(b)** Nitrate concentrations; and **(c)** Phosphate concentrations.

Why is phosphorus limiting in Chinese coastal waters?

Preliminary observations on nutrient concentrations are available for two estuaries in China. The Changjiang estuary shows a steep, decreasing gradient in nitrate, phosphate, and silicate concentrations as one proceeds from the river, through the estuary, and out to the open sea (Edmond et al. 1985; Shi et al. 1986; Sun et al. 1986). For June 1980, over the salinity range 0–32‰, the ranges of nitrate, phosphate, silicate, and ammonium concentrations were, 65–15, 0.7–0.0, 105–10, and 2–2 μM, respectively (Edmond et al. 1985). The N:P ratio over the salinity range of 0-32‰ was about 90:1–150:1. In November, nitrate and phosphate in the river were 45 and 0.3 μM, respectively, yielding a N:P ratio of about 140:1. In the summer, vigorous turbulence in the main channel of the inner estuary maintains high concentrations of suspended material (greater than 1 000 mg·kg^{-1}; Milliman et al. 1985) in the surface layers, which suppresses biological activity. Phytoplankton blooms occur only on the inner shelf at salinities greater than 20‰.

Edmond et al. (1985) concluded that phosphate rather than nitrate was the limiting nutrient for biological productivity on the shelf, the reverse of the normal situation. They also concluded that the unusually high nitrate concentrations in the river were derived from agricultural sources. The yearly input of nitrate per square kilometre of drainage area for the Changjiang River is twice as much, whereas phosphate is half as much, as that of the Amazon River (Edmond et al. 1985). Measurements of nitrate farther offshore from the estuary reveal that the influence of nitrate input from the river extends out to the middle of the East China Sea (i.e., >500 km) (Shi et al. 1986). Beyond the estuary, primary productivity is high, ranging from 20–2 240 mg C·m^{-3} (Chai 1986).

The Huanghe (Yellow) River estuary in the southwest portion of the Bohai Sea was studied in July 1984 and in May and late August 1985 (Lu et al. 1985; Chen et al. 1986; Turner et al. 1987). Again, nitrate, phosphate, and silicate concentrations decrease dramatically as one moves from the river to the offshore region. The ranges in concentrations were as follows: nitrate, 140–1; phosphate, 1.0–<0.05; and silicate, 170–5 μM. The nitrate:phosphate ratios are shown in Fig. 4 as a steep gradient in contour lines ranging from >80:1 in the estuary to 10:1 in the Bohai Sea (Lu et al. 1985; Zou et al. 1985). Evidence indicating that nitrogen is most probably the limiting nutrient in areas beyond the estuary comes from the observation that cultivation of the seaweed *Laminaria japonica* is enhanced by adding nitrate rather than phosphate in this area (Tseng et al. 1955).

Moving offshore to the east, toward Taiwan, N:P ratios are generally less than 10:1 with nitrate values generally <5 μM and phosphate about 0.6 μM (Hung and

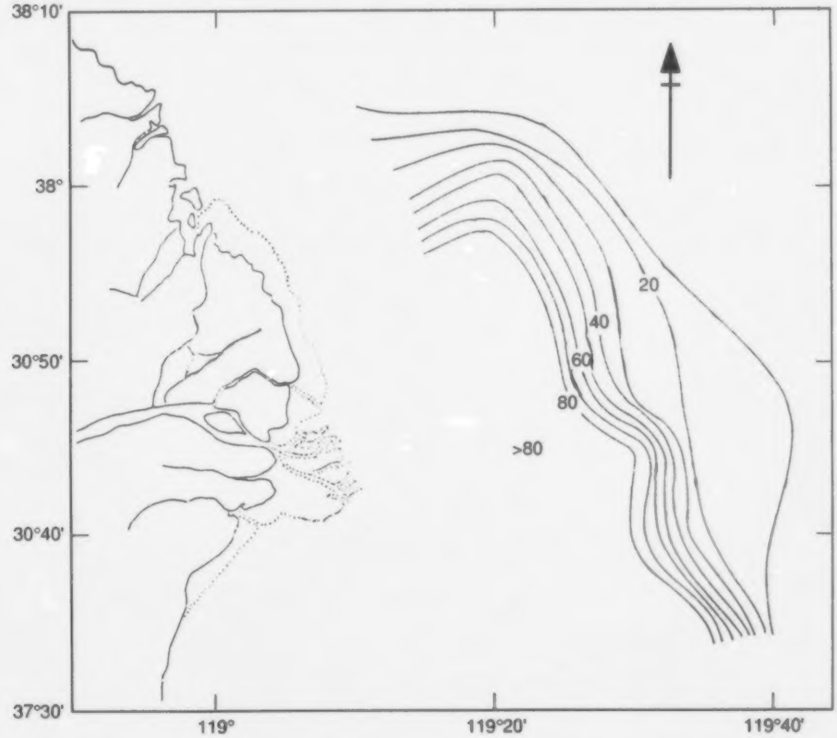

Fig. 4. Contours of nitrate:phosphate ratios (by atoms) in surface waters of the Huanghe River estuary (from Lu et al. 1985).

Tsai 1980; Hung et al. 1982, 1986). Similarly, off Hong Kong, N:P ratios were also <10:1, indicating that nitrogen was most likely limiting phytoplankton growth (Wear et al. 1984; Chiu et al. 1985).

Therefore, two of China's large rivers, the Changjiang and the Huanghe, appear to act like giant nutrient pumps, delivering excess nitrogen, relative to phosphorus, to coastal waters. At the outer edge of the estuary, therefore, phytoplankton growth is potentially limited by phosphorus, whereas well beyond the influence of the nutrients from the river (often >500 km offshore) nitrogen is limiting (the normal situation). In the inner part of the estuary, a high suspended load results in very turbid waters; therefore, primary productivity is light-limited and nutrients are never exhausted.

North of Xiamen Bay, where the mesocosm experiments were conducted, the Minjiang River (another large river in China) forms another large estuary. Nutrient distribution in the estuary has not been studied, but current measurements suggest that current flow from this area is often south along the coast. Therefore, nutrient input from this river could conceivably affect the Xiamen Bay area and could account for high nitrate relative to phosphate concentrations and the high N:P ratios (Fig. 3).

Similar observations have been made in the North Sea where polluted rivers have high N:P ratios (28:1–63:1); this is in contrast to unpolluted rivers where N:P ratios range from 8:1 (Amazon and Zaire rivers) to 21:1 (Niger River) (Wollast 1983). The nutrient-rich river water originating from the Scheldt, Meuse, and Rhine rivers mixes with seawater in such a way that 50% of the river discharge is transported in a 15-km wide strip along the Dutch and Belgian coasts. Therefore, the effects of nutrient input from rivers are often observed some distance from the estuary. At the edge of this 15-km strip, phosphate and silicate may limit phytoplankton growth in the spring.

Another explanation for phosphorus being limited in estuarine coastal waters is that phosphate may adsorb to suspended particles and sink to the sediments, carrying phosphate with it. This has been observed in the Changjiang estuary (Edmond et al. 1985) and in other large, turbid rivers, such as the Amazon River (Chase and Sayles 1980).

It has also been suggested that Chinese farmers use less phosphorus fertilizer because it is expensive and that agricultural land along the coast is low in phosphorus. This would help explain the low amount of phosphorus relative to nitrogen in the runoff.

Cyanobacteria or blue–green algae may be abundant in these waters. If they fix N_2, this would result in the depletion of phosphate. Dense blooms of *Trichodesmium* (*Oscillatoria*) have been reported in the Kuroshio current, but not in the East China Sea (Nishimura 1983).

Phosphorus limitation in estuarine areas along the coast of China is not totally unique (Smith 1984). Some estuaries along the east coast of the United States notably upper Chesapeake Bay, Hudson River, and Apalachicola Bay (Florida), are phosphorus-limited (Taft and Taylor 1975; Myers and Iverson 1981; Boynton et al. 1982). In Australia, the Peel–Harvey estuaries are phosphorus-limited in the winter and nitrogen-limited in the summer (McComb et al. 1981). Other larger areas, such as the Mediterranean Sea, have also been suggested as being phosphorus-limited (Berland et al. 1980; Maestrini and Kossut 1981; Azov 1986).

Phosphorus-limited growth of the macrophytes *Sargassum* spp and *Gracilaria tikvahiae* has been reported in the western North Atlantic off Florida (Lapointe 1986, 1987). Another macrophyte, *Macrocystis pyrifera*, has been reported as showing phosphorus-limited growth in the winter off California (Manley and North 1984).

The discharge of phosphorus and nitrogen transported by the Rhine River has increased sixfold since 1930. This increase in nitrogen and phosphorus and the essentially constant discharge of silicate has resulted in diatoms being silicate-limited, whereas flagellates are phosphorus-limited (Fransz and Verhagen 1985).

During blooms in Norwegian waters, phytoplankton communities are phosphorus-limited in fresh and brackish waters, and balanced or nitrogen-limited in marine waters. This results from the high N:P ratio in fresh or brackish water (<100:1) relative to the lower N:P ratio (12:1–16:1) in marine waters (Sakshaug and Olsen 1986).

Other estuaries in the United States are clearly nitrogen-limited, with N:P values <10:1 (Nixon 1981; Boynton et al. 1982; Pilson 1985). In these estuaries, dentrification has been reported to be a very important process where up to 50% of the nitrate may be lost to the atmosphere as N_2 (Pilson 1985; Smith et al. 1985).

Phosphorus versus nitrogen limitation — does it matter?

In most temperate and tropical areas, phytoplankton growth frequently becomes either phosphorus-limited or nitrogen-limited during part of the year. Therefore, it seems logical that cells that are already naturally stressed by nutrient limitation may be more sensitive to a pollutant (secondary stress) than cells that are healthy. It has been shown by Cloutier-Mantha and Harrison (1980) that ammonium-limited *Skeletonema* exposed to Hg had a higher Ks value for ammonium uptake and the cells could not deplete ammonium in the medium below 1 μM. Normal cells can take ammonium down to undetectable levels.

The question of whether phosphorus-limited cells are more sensitive to pollutants than nitrogen-limited cells has not been well studied. Only one study has attempted to examine this question and Karydis (1981) found that phosphorus-starved cells of *Skeletonema* were more sensitive to oil pollution than nitrogen-starved cells. This interesting question certainly deserves further study.

During normal growth, many phytoplankton store phosphorus intracellularly in polyphosphate granules (Jensen et al. 1986; Sicko-Goad and Lazinsky 1986). Recent studies have shown that heavy metals, such as Pb, are sequestered by these granules. During phosphorus limitation, the polyphosphate granules are degraded and the sequestered heavy metals are liberated internally. This may have adverse effects on cells that are already nutrient stressed. Observations such as this may help explain why phosphorus-limited cells appear to be more sensitive to pollutants than nitrogen-limited cells.

The second consequence of whether the ecosystem is phosphorus-limited or nitrogen-limited involves potential species changes if eutrophication is controlled or altered in the future. For example, a low N:P ratio (i.e., nitrogen limitation) is known to favour cyanobacteria, whereas phosphorus limitation may favour diatoms (Smith 1983). Therefore, if the N:P ratio in the runoff and sewage was altered in the future and the N:P ratio decreased, China's coastal waters would tend toward nitrogen limitation with potential shifts in the dominant species of phytoplankton. These species shifts could affect higher trophic levels.

Finally, most models of marine ecosystems are nitrogen based (Parsons and Kessler 1986b) or carbon based. For estuarine areas along the coast of China, nitrogen-based models, such as the one used to simulate mesocosm experiments in Canada (Parsons and Kessler 1986a), must be changed to a phosphorus-based model.

Acknowledgments

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Azov, Y. 1986. Seasonal patterns of phytoplankton productivity and abundance in nearshore oligotrophic waters of the Levant Basin (Mediterranean). Journal of Plankton Research, 8, 41–53.

Berland, B.R., Bonin, D.J., Maestrini, S.Y. 1980. Azote ou phosphore? Considerations sur le "paradoxe nutritionnel" de la mer mediterranée. Oceanologica Acta, 3, 135–141.

Boynton, W.R., Kemp, W.M., Keefe, C.W. 1982. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. *In* Kennedy, V.S., ed., Estuarine comparisons. Academic Press, New York, NY, USA. 69–89.

Chai, X. 1986. Comprehensive survey and research report on the water areas adjacent to the Changjiang River estuary and Chejudo Island: 5. Marine biology — the distribution of the concentration of the chlorophyll *a* and the estimation of the primary production. Journal of Shandong College of Oceanology, 16, 1–26.

Chase, E.M., Sayles, F.F. 1980. Phosphorus in suspended sediments of the Amazon River. Estuarine and Coastal Marine Science, 11, 383–391.

Chen, S., Gu, Y., Liu, M., Zhang, M., Yang, S., Li, J. 1986. The distribution of nutrients in the Huanghe estuary and adjacent sea. Report from a symposium of joint research on the geochemistry of trace elements in the Huanghe estuary, July 1986. Unpublished.

Chiu, M.T.L., Chung, R.T., Luk, R.W.K. 1985. Chemical hydrographic observations of the southern and eastern waters of Hong Kong. Asian Marine Biology, 2, 37–46.

Cloutier-Mantha, L., Harrison, P.J. 1980. Effects of sublethal concentrations of mercuric chloride on ammonium-limited *Skeletonema costatum*. Marine Biology, 56, 219–231.

Edmond, J.M., Spivack, A., Grant, B.C., Hu, M.H., Chen, Z., Chen, S., Zeng, X. 1985. Chemical dynamics of the Changjiang estuary. Continental Shelf Research, 4, 17–36.

Fransz, H.G., Verhagen, J.H.G. 1985. Modelling research on the production cycle of phytoplankton in the southern bight of the North Sea in relation to riverborne nutrient loads. Netherlands Journal Sea Research, 19, 241–250.

Goldman, J.C. 1975. Identification of nitrogen as a growth limiting nutrient in wastewaters and coastal marine waters through continuous culture algal assays. Water Research, 10, 97–104.

Grice, G.D, Reeve, M.R. 1982. Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 430 pp.

Harrison, P.J., Fulton, J.D., Taylor, F.J.R., Parsons, T.R. 1983. Review of the biological oceanography of the Strait of Georgia: pelagic environment. Canadian Journal of Fisheries and Aquatic Sciences, 40, 1 064–1 094.

Harrison, P.J., Cochlan, W.P., Acreman, J.C., Parsons, T.R., Thompson, P.A., Dovey, H.M. 1986. The effects of crude oil and Corexit 9527 on marine phytoplankton in an experiment enclosure. Marine Environmental Research, 18, 93–109.

Hung, T., Tsai, C.C.H. 1980. Chemical nutrients and apparent oxygen utilization in the sea water surrounding Taiwan. Bulletin of the Institute of Chemistry, Academia Sinica, 27, 33–43.

Hung, T., Chuang, A., Chou, B., Cheng, T. 1982. Relationships among POC, ATP, chlorophyll *a* and primary productivity in the sea water along Taiwan's western coast. Acta Oceanographica Taiwanica, 13, 109-123.

Hung, T., Tsai, C.C.H., Wu, S., Chuang, A. 1986. Study on biomass and primary productivity along the southwestern coast of Taiwan. Acta Oceanographica Taiwanica, 16, 8–26.

Jensen, T.E., Rachlin, J.W., Jan, V., Warkentine, B.E. 1986. Heavy metal uptake in relation to phosphorus nutrition in *Anabaena variabilis* (Cyanophyceae). Environmental Pollution, 42, 261–271.

Karydis, M. 1981. The toxicity of crude oil for the marine alga *Skeletonema costatum* (Greville) Cleve in relation to nutrient limitation. Hydrobiologia, 85, 137–143.

Lapointe, B.E. 1986. Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum fluitans* (Phaeophyceae) in the western North Atlantic. Deep-Sea Research, 33, 391–399.

______ 1987. Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. Marine Biology, 93, 561–568.

Lu, X., Zhu, C., Zhang, A., Shi, Z. 1985. The characteristics of nutrient distribution in the southwest Bohai Sea and Huanghe River estuary in summer. Journal of Shandong College of Oceanology, 15, 146–158.

Maestrini, S.Y., Kossut, M. 1981. In situ cell depletion of some marine algae enclosed in dialysis sacks and their use for the determination of nutrient-limiting growth in Ligurian coastal waters (Mediterranean Sea). Journal of Experimental Marine Biology and Ecology, 50, 1–19.

Manley, S.L., North, W.J. 1984. Phosphorus and the growth of juvenile *Macrocystis pyrifera* (Phaeophyta) sporophytes. Journal of Phycology, 20, 389–393.

McComb, A.J., Atkins, R.P., Birch, P.B., Gordon, D.M., Lukatelich, R.J. 1981. Eutrophication in the Peel–Harvey estuarine system, western Australia. *In* Neilson, B.J., Cronin, L.E., ed., Estuaries and nutrients. Humana Press, Clifton, NJ, USA. 323–338.

Milliman, J.D., Shen, H-T., Yang, Z-S., Meades, R.H. 1985. Transport and deposition of river sediment in the Changjiang estuary and adjacent continental shelf. Continental Shelf Research, 4, 37–45.

Myers, V.B., Iverson, R.I. 1981. Phosphorus and nitrogen limited phytoplankton productivity in northeastern Gulf of Mexico coastal estuaries. *In* Neilson, B.J., Cronin, L.E. ed., Estuaries and nutrients, Humana Press, Clifton, NJ, USA. 569–582.

Nishimura, S. 1983. Okhotsk Sea, Japan Sea, East China Sea. *In* Ketchum, B.H., ed., Ecosystems of the world — 26: Estuaries and enclosed seas. Elsevier, New York, NY, USA. 375–401.

Nixon, S.W. 1981. Remineralization and nutrient cycling in coastal marine ecosystems. *In* Neilson, B.J., Cronin, L.E., ed., Estuaries and nutrients. Humana Press, Clifton, NJ, USA. 111–138.

Parsons, T.R., Kessler, T.A. 1986a. An ecosystem model analysis of the effect of mine tailings on the euphotic zone of a pelagic ecosystem. Acta Oceanologica Sinica, 5, 425–436.

______ 1986b. Computer model analysis of pelagic ecosystems in estuarine waters. *In* Skreslet, S. ed., The role of freshwater outflow in coastal marine ecosystems. Springer-Verlag, Berlin, Germany. NATO ASI Series, Vol. G7, 161–181.

Parsons, T.R., Thompson, P., Wu, Y., Lalli, C.M., Hou, S., Xu, H. 1986. The effect of mine tailings on the production of plankton. Acta Oceanologica Sinica, 5, 417–434.

Pilson, M.E.Q. 1985. Annual cycles of nutrients and chlorophyll in Naragansett Bay, Rhode Island. Journal of Marine Research, 43, 849–873.

Ryther, J.H., Dunstan, W.M. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science, 171, 1 008–1 013.

Sakshaug, E., Olsen, Y. 1986. Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition. Canadian Journal of Fisheries and Aquatic Science, 43, 389–396.

Shi, Z, Sun, B., Wan, Y. 1986. Comprehensive survey and research report on the water areas adjacent to the Changjiang River estuary and Chejudo Island: 4 — Marine chemistry — inorganic nitrogen in seawater. Journal of Shandong College of Oceanology, 16, 189–207.

Sicko-Goad, L., Lazinsky, D. 1986. Quantitative ultrastructural changes associated with lead-coupled luxury phosphate uptake and polyphosphate utilization. Archives of Environmental Contamination and Toxicology, 15, 617–627.

Smith, C.J., DeLaune, R.D., Patrick, W.H., Jr. 1985. Fate of riverine nitrate entering an estuary. I: Dentrification and nitrogen burial. Estuaries, 8, 15–21.

Smith, S.V. 1984. Phosphorus versus nitrogen limitation in the marine environment. Limnology and Oceanography, 29, 1 149–1 160.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favour dominance by blue–green algae in lake phytoplankton. Science, 221, 669–670.

Sun, B., Yu, S., Hao, E. 1986. Comprehensive survey and research report on the water areas adjacent to the Changjiang River estuary and Chejudo Island: 4 — Marine chemistry — soluble phosphate in seawater. Journal of Shandong College of Oceanology, 16, 172–182.

Taft, J.L., Taylor, W.R. 1975. Phosphorus dynamics in some coastal plain estuaries. *In* Wiley, M., ed., Estuarine processes (vol 1). Academic Press, New York, NY, USA. 79–89.

Tseng, C.K., Sun, K.Y., Yu, C.Y. 1955. Studies on fertilizer application in the cultivation of haitai. Acta Botanica Sinica, 4, 375–392.

Turner, R.E., Rabalais, N.N., Zhang, Z. 1987. Plankton dynamics in the Yellow River plume. Eos, 68, 1 735.

Wear, R.G., Thompson, G.B., Stirling, H.P. 1984. Hydrography, nutrients and plankton in Tolo Harbour, Hong Kong. Asian Marine Biology, 1, 59–75.

Wollast, R. 1983. Interactions in estuaries and coastal waters. *In* Bolin, B., Cook, R.B., ed., The major biogeochemical cycles and their interactions. SCOPE (Scientific Committee on Problems of the Environment). 385–410.

Zou, J., Dong, L., Qin, B. 1985. Preliminary studies on eutrophication and red tide problems in Bohai Bay. Hydrobiologia, 127, 27–30.

Effects of Heavy Metals and Sediment Pollutants on Phytoplankton and Primary Productivity in an Enclosed Ecosystem

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An enclosed ecosystem experiment was carried out in Xiamen, People's Republic of China, in 1985. A mixture of heavy metals (Cd, Cu, Pb, Zn, and Hg) was added in two different concentrations to three bags. Sediment from the waste outfall of Xinglin Chemical Plant, Xiamen, was added in two different concentrations to three other bags. Neither sediment nor metal mixtures were added to the control bag.

Phytoplankton in the control bag were dominated by diatoms (mainly Skeletonema costatum *and* Cylindrotheca closterium*) and the bloom was terminated by phosphorus limitation. Zooplankton were dominated by two herbivores,* Acartia pacifica *and* Paracalanus parvus, *which increased near the end of the experiment in the control bag and exerted considerable grazing pressure on the diatoms. In the bag that received a low concentration of the heavy metal mixture, zooplankton decreased during the early part of the experiment. This reduction in grazing pressure allowed diatoms and, consequently, chlorophyll* a *and primary productivity to increase faster and bloom earlier than in the control. The bag receiving the low concentration of sediment was intermediate between the control and the low-metal concentration bag. In the high-metal bag, zooplankton growth was the most severely inhibited of all the bags and phytoplankton were also stressed as they bloomed much later than in the control. The number of diatoms, the chlorophyll* a *concentration, and the primary productivity were higher in the high-metal bag than in the low-metal bag near the end of the experiment because of a reduction in the number of zooplankton and, hence, the grazing pressure in the high-metal bag. The bags receiving the high concentration of sediment did not behave noticeably differently than the bags receiving a lower concentration of sediment. Centric diatoms were dominant in the control bag whereas pennate diatoms were dominant in the polluted bags, suggesting that centric diatoms were more sensitive to the pollutants than pennate diatoms. Euglenophytes increased only in the bag with the highest* *concentration of heavy metals, demonstrating a strong tolerance by this algal group to heavy metals.*

Recently, enclosed ecosystem experiments have been considered to be the best approach to study ecosystem structure, function, and dynamic processes (Hollibaugh et al. 1980; Thomas et al. 1980). Several experiments on the effect of heavy metals on phytoplankton have been conducted in enclosed ecosystems. Thomas and Seibert (1977) and Thomas et al. (1977) studied the effects of Cu and Hg on phytoplankton biomass, primary productivity, dominant species, and the diversity index. Their results showed that centric diatoms were more sensitive to Cu and Hg than pennate diatoms, which led to changes in species succession and community structure in the ecosystem.

In China, some small-scale experiments similar to the enclosed ecosystem experiments were conducted in Qingdao and Dailian harbours in 1975, 1976, and 1978. Under natural conditions, domestic and imported crude oils were used to study their effects on phytoplankton species and biomass. Some early results from these experiments have been used to modify the experimental approaches used to study the effects of pollutants in enclosed ecosystems. An enclosed ecosystem experiment was carried out in 1985 at the Third Institute of Oceanography, Xiamen, by Canadian and Chinese scientists.

In this paper, primary productivity, phytoplankton species composition, and fluctuations in the abundance of planktonic diatoms and their relationships with pollutants, zooplankton, and other environmental factors are discussed.

Materials and methods

The experiment was performed from 18 April to 8 May 1985 in the pool on the seashore near the Third Institute of Oceanography, Xiamen. The pool was 20 m long, 10 m wide, and 5 m deep. Above the pool was a fibreglass roof that reduced the incident light intensity by 50%. The pool contained nine polyethylene bags, each 2 m in diameter and 4 m long (the top 3 m was cylindrical and the bottom 1 m was conical). The opening of the bag was attached to a wooden frame, which supported the bags. The bags contained 10 m^3 of seawater, which was pumped using a diaphragm pump, from 150 m offshore. The filling process was completed within 5 h. Additional experimental details are presented by Wu J. et al. (this volume).

A heavy metal mixture of Cd, Cu, Hg, Zn, and Pb was added to three bags. Polluted sediment obtained from the waste outfall of Xinglin Chemical Plant, Xiamen, was added to three other bags.

The bags in the pool were arranged at random. C1 and C2 were the controls (i.e., no metal or sediment additions). M1 and M2 were duplicates to which heavy metals were added at low concentrations, whereas M3 contained heavy metals in higher concentrations (Table 1). S1 and S2 were duplicates to which sediment (112 g dry weight$\cdot L^{-1}$) was added. S3 contained the most sediment (1 120 g dry weight$\cdot L^{-1}$). Samples were taken from all of the bags to establish initial (background) values before nutrients and pollutants were added. On day zero, nutrients were added at a ratio of nitrate:silicate:phosphate of 5:5:0.5 μg-at$\cdot L^{-1}$ to the nine bags. Samples were taken on days 1, 3, 5, 8, 11, 14, 17, and 20. An integrated

Table 1. Added and range of measured concentrations ($\mu g \cdot L^{-1}$) of dissolved heavy metals in bags. (Background concentrations for Xiamen Harbour are also listed.)

			Bag						Xiamen
Metal			C1	M1	M3	S1	S2	S3	Harbour
Cu	Measured	High	0.47	3.28	8.48	0.78	0.79	1.89	0.4
		Low	0.30	1.40	5.14	0.38	0.42	1.37	—
	Added		0.0	3.5	10.0	0.0	0.0	0.0	—
Cd	Measured	High	0.039	1.16	7.15	0.053	0.058	0.139	0.02
		Low	0.025	0.81	5.32	0.027	0.029	0.074	—
	Added		0.0	1.0	5.0	0.0	0.0	0.0	—
Hg	Measured	High	0.003	0.066	0.371	0.004	0.004	0.004	0.02
		Low	0.001	0.022	0.142	0.001	0.002	0.002	—
	Added		0.0	0.2	1.0	0.0	0.0	0.0	—
Pb	Measured		—	—	—	—	—	—	0.03
	Added		0.0	0.3	1.5	0.0	0.0	0.0	—
Zn	Measured		—	—	—	—	—	—	0.7
	Added		0.0	3.5	17.5	0.0	0.0	0.0	—

Note: C1 = control, M1 and M3 = low and high concentration of metal mixture added, S1 and S2 = duplicates, low sediment addition, S3 = high sediment addition.

seawater sample from 0–3 m was obtained using a diaphragm pump and then stored in a large plastic container. A 500-mL aliquot was taken and Lugol's solution was added to preserve the phytoplankton. Identification of phytoplankton species and counting were accomplished using an inverted microscope (× 320 magnification). The preserved samples were shaken well, poured into a settling chamber (5 mL), and settled for 5 h before they were counted. At least 100 cells of the major algal groups or species were counted. Counting precision was about ±10%. The dominant group or species was defined as the one whose cell numbers amounted to 10% or more of the total cell numbers. Microflagellates were not identified to genus or species levels, but were counted as size groups (1-5 μm, 6–10 μm, and 11–20 μm).

Data on nutrients, light intensity, temperature, salinity, and zooplankton were collected simultaneously. Methods for measuring these parameters have been described elsewhere (Harrison et al., this volume; Wu J. et al., this volume). Bags C2 and M2 floated to the surface several days after launching; consequently, data for these two bags will not be discussed.

Chlorophyll *a* was determined by in vitro fluorescence (Parsons et al. 1984) and primary productivity was measured following the ^{14}C technique (Parsons et al. 1984). About 5 μCi (185 kBq) $NaH^{14}CO_3$ was added to a 100-mL seawater sample and incubated for 4 h in situ at 1.5 m in the appropriate bag. At the end of the incubation period, the sample was filtered through a 0.8-μm Millipore filter, placed in a scintillation vial to which acid had been added, and counted in a liquid scintillation counter.

Results and discussion

Control bag

Phytoplankton (mainly diatoms) increased slowly until day 8, at which time they increased rapidly (Fig. 1). Environmental factors considered to be responsible for phytoplankton fluctuations in the control bag were light, nutrients, and grazing by zooplankton. This experiment demonstrated that the slow growth of diatoms during the early part of the experiment was probably due to reduced light intensity. Incident light intensity was reduced 50% by the fibreglass roof, and was reduced even further by cloudy and rainy conditions during the early part of the experiment. This resulted in raising the compensation depth to 2 m or even shallower. The diatom bloom was dominated by *Skeletonema costatum* and *Cylindrotheca closterium*, which consumed most of the nutrients.

By day 11, nitrate plus nitrite concentrations were 60% of their initial value, the concentration of phosphorus was near zero, and the concentration of silicate was 71% of its initial value (Table 2). Therefore, phosphate exhaustion was responsible for the decrease in phytoplankton during the latter part of the experiment (Harrison et al., this volume). Zooplankton, represented mainly by the herbivores *Acartia pacifica* and *Paracalanus parvus*, changed with phytoplankton cell numbers. Therefore, grazing on phytoplankton may have been important near the end of the experiment. The number of microflagellates remained low throughout the experiment. The reason is not clear, but it could be due to zooplankton grazing or the inability of microflagellates to outcompete diatoms.

Low metal or sediment additions

In bags M1, S1, and S2 (Figs 2–4), diatoms increased until day 5; this increase was not observed in the control. These results indicate that low levels of pollutants did not stress phytoplankton growth, but stimulated it or reduced grazing pressure. At the same time, numbers of *Acartia pacifica* and *Paracalanus parvus* in bag M1 decreased, whereas the numbers of these two herbivores in the control doubled. Therefore, the reason diatom abundance in M1 during the early part of the experiment was larger than that in C1 might be due to the decrease in herbivorous zooplankton, and the subsequent reduction in grazing pressure.

Microflagellates also increased markedly, relative to the control, perhaps due to reduced grazing pressure or a reduction in the competitive ability of the pollution-stressed diatoms. The effect of a possible reduction in grazing pressure was also revealed by an earlier increase in chlorophyll *a* in M1 and stimulation in primary productivity compared with the control (Fig. 5). The time when the phytoplankton maximum occurred in M1 (day 17) was not only 3–6 d later than for C1 but it was also far less than the maximum of C1 (about 1/60th).

The change in phytoplankton numbers in S1 and S2 was intermediate between M1 and the control bag. As the amount of nutrients consumed in S1 and S2 was less than in M1 (which had a lower phytoplankton biomass), this suggests that additional nutrients may have come from the sediments. The maximum cell number in S1 and S2 was far lower (ca. 85% lower) than that in the control. Zooplankton abundance was also intermediate between the control and M1. These results

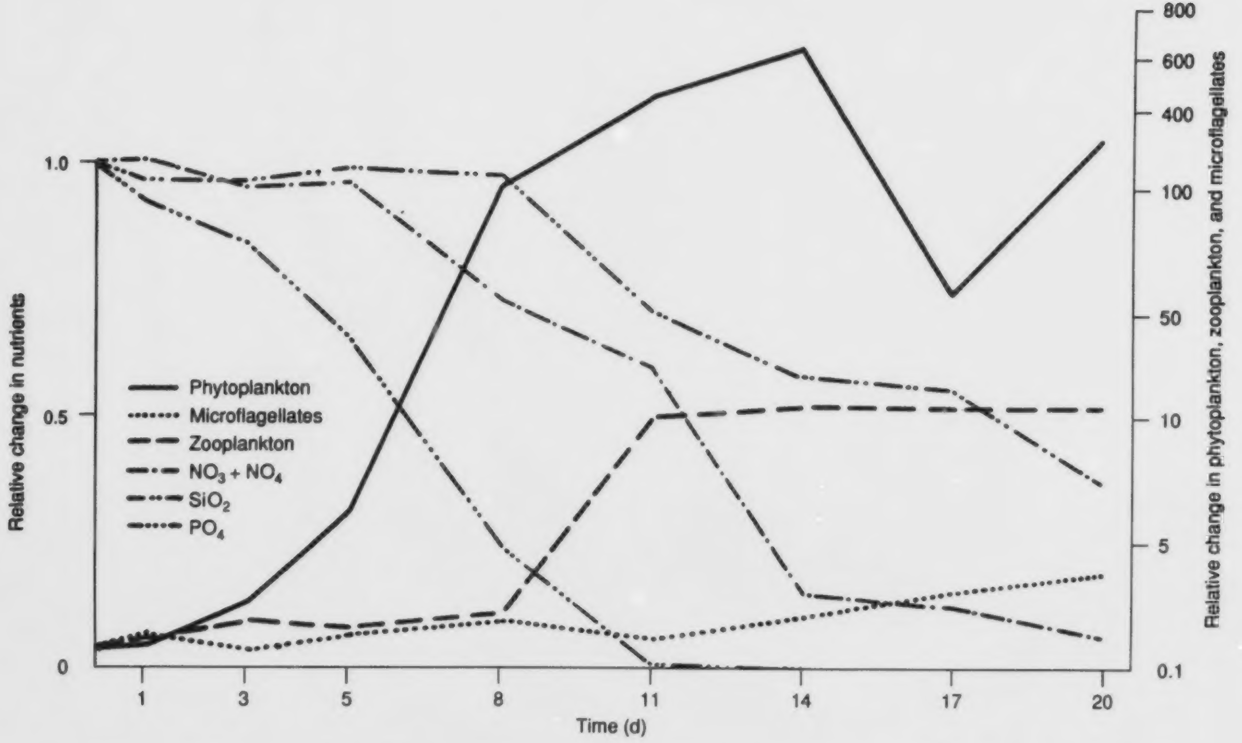

Fig. 1. Changes in phytoplankton (mainly diatoms), microflagellates, zooplankton, and nutrients (nitrate, phosphate, and silicate) in the control bag C1 expressed as a ratio relative to initial concentrations.

Table 2. Change in concentration (μM) of NO_3 + NO_2, PO_4, and SiO_4 in the bags over time.

		Day								
Nutrient	Bag	0	1	3	5	8	11	14	17	20
NO_3 + NO_2	C1	24.0	24.1	22.9	23.1	17.6	14.4	3.8	3.0	1.6
	M1	23.7	23.0	23.1	23.0	14.3	10.5	4.2	6.7	6.1
	M3	24.9	24.9	24.8	25.7	20.8	17.2	10.6	12.8	2.2
	S1	25.1	23.8	25.2	24.1	17.6	13.2	8.3	15.7	6.1
	S2	24.5	22.9	23.8	23.4	19.6	14.9	16.0	20.8	8.6
	S3	25.7	24.7	25.6	25.3	16.5	11.1	11.2	19.8	6.8
PO_4	C1	0.76	0.70	0.64	0.50	0.19	0.01	UD	UD	UD
	M1	0.79	0.67	0.57	0.55	0.12	0.01	0.07	0.06	UD
	M3	0.81	0.72	0.65	0.66	0.54	0.31	0.01	0.06	UD
	S1	0.82	0.75	0.89	0.71	0.42	0.36	0.18	0.26	0.20
	S2	0.83	0.79	0.71	0.76	0.57	0.52	0.46	0.38	0.24
	S3	0.97	1.01	1.05	0.97	0.41	0.38	0.47	0.39	0.37
SiO_4	C_1	56.5	54.8	54.3	56.0	55.5	40.2	33.1	31.2	21.0
	M1	47.1	47.6	47.1	51.2	49.0	46.5	44.9	41.3	34.1
	M3	52.9	54.0	51.8	60.3	53.9	47.4	50.4	43.8	33.2
	S1	50.9	51.2	50.9	55.8	56.6	48.2	46.0	38.9	27.9
	S2	50.9	51.5	50.7	53.0	52.0	46.3	42.7	37.6	28.2
	S3	54.5	55.9	54.5	53.3	54.1	47.6	50.1	38.3	29.6

Note: UD, undetectable; C1 = control, M1 and M3 = low and high concentration of metal mixture added, S1 and S2 = duplicates, low sediment addition, S3 = high sediment addition.

suggest that phytoplankton and zooplankton in S1 and S2 were also affected by pollution stress from the sediment, but the stress was smaller than that in M1. In fact, the heavy metal concentrations in M1 (Cu, Cd, and Hg) were greater than those in S1 and S2 (Cu was 3.8 times, Cd was 21.4 times, and Hg was 13 times, Table 1).

High metal or sediment additions

In the bags with higher concentrations of pollutants (M3, high concentration of heavy metals; high concentration of sediments), diatoms were most strongly stressed during the first 3 d and cell numbers declined by 40–90% (Figs 6 and 7). The two species of herbivorous zooplankton also decreased by 60–70% of their initial value. The major factor responsible for the decrease in phytoplankton and zooplankton was the pollutant stress (Cu in M3 was 16.7 times, Cd was 185 times, and Hg was 81 times higher than the concentration in the control). Microflagellates remained low during the experiment, but slowly increased near the end.

Diatom cell numbers in S3 decreased by 40–70% of their initial value before day 3, indicating less pollutant stress than in M3 (Cu, Cd, and Hg concentrations in S3 were 2.8, 2.2, and 1.5 times greater than in the control). It was surprising to find that the number of phytoplankton in M3 (most polluted bag) was far greater than

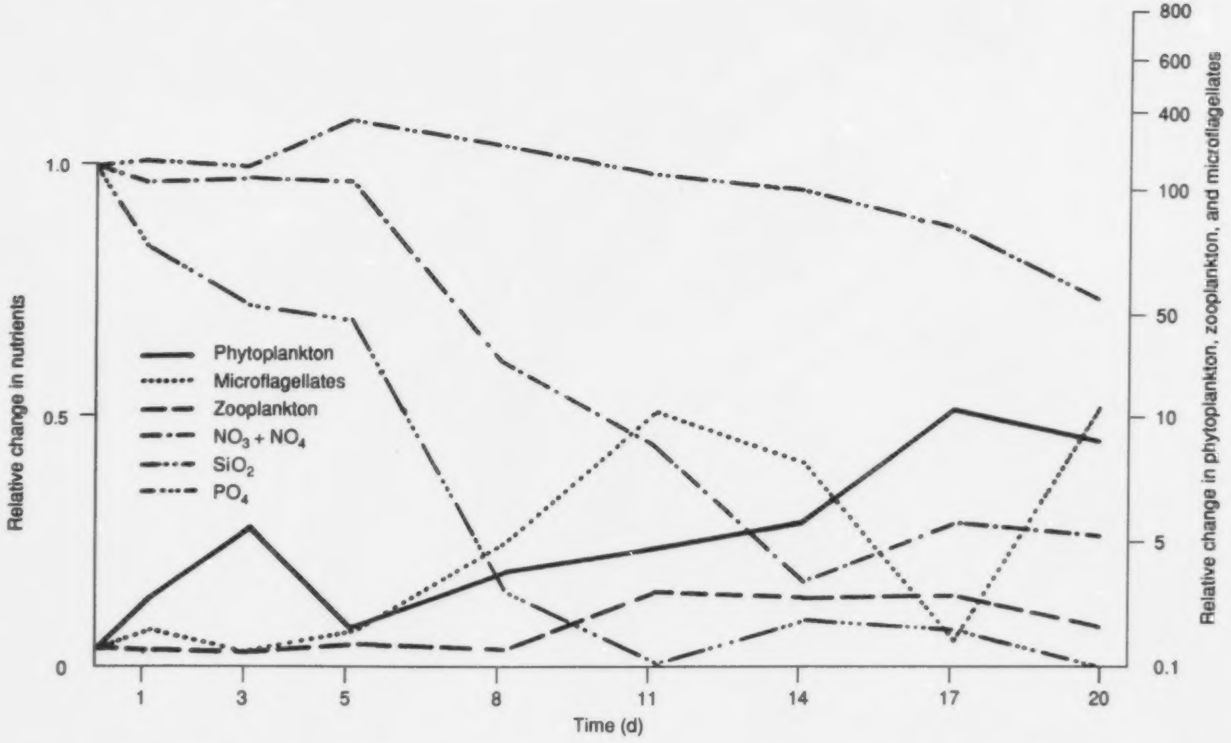

Fig. 2. Changes in phytoplankton (mainly diatoms), microflagellates, zooplankton, and nutrients (nitrate, phosphate, and silicate) in bag M1 (low concentration of heavy metal mixture) expressed as a ratio relative to concentrations in control bag C1.

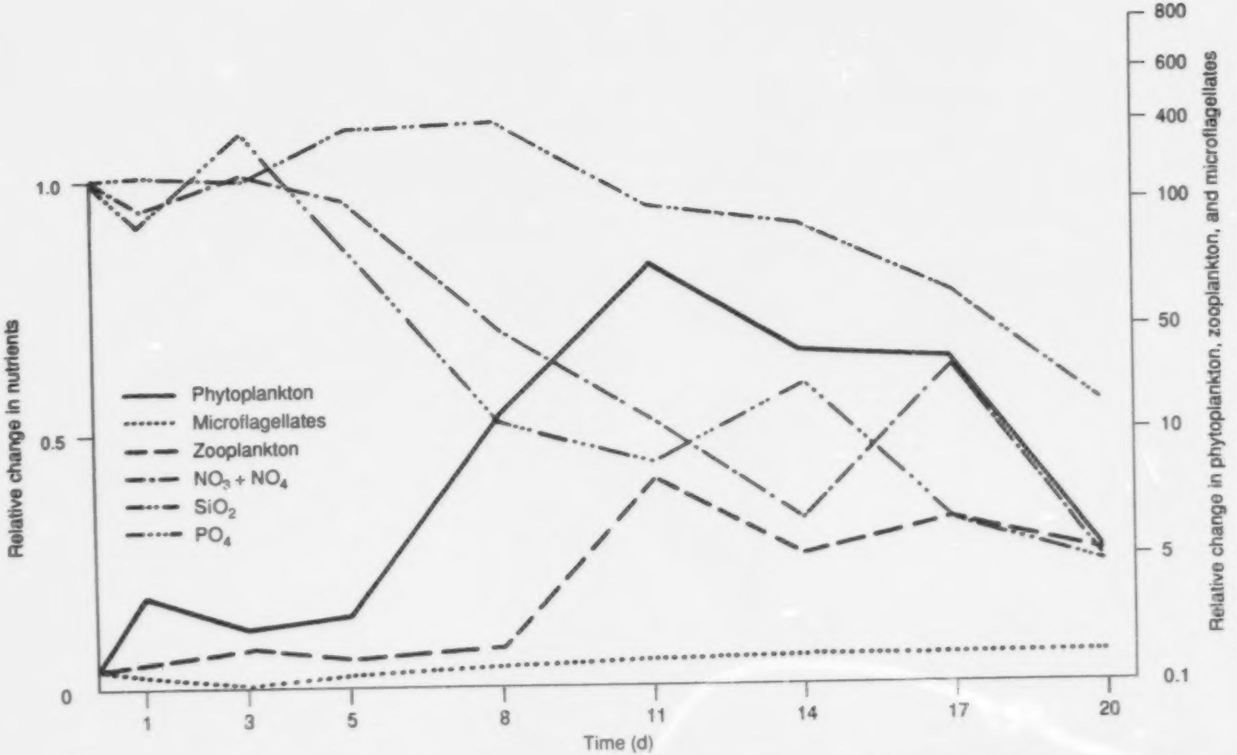

Fig. 3. Changes in phytoplankton (mainly diatoms), microflagellates, zooplankton, and nutrients (nitrate, phosphate, and silicate) in bag S1 (low concentration of sediment) expressed as a ratio relative to concentrations in control bag C1.

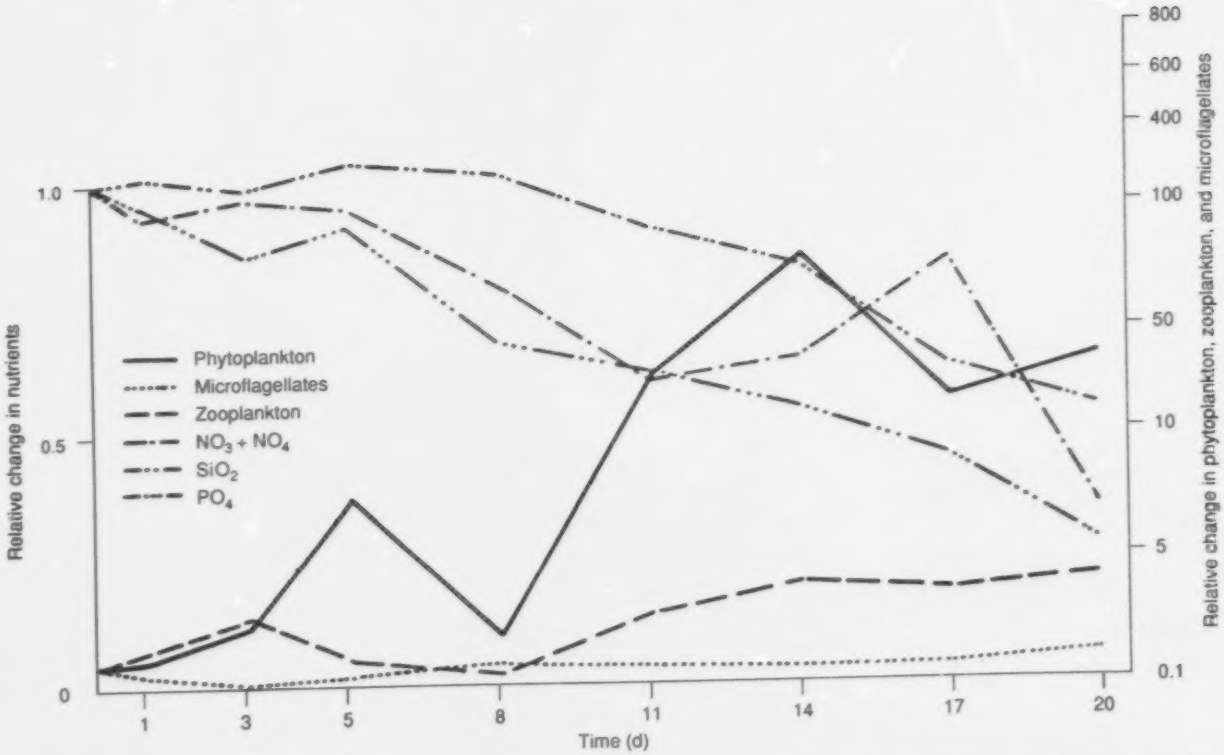

Fig. 4. Changes in phytoplankton (mainly diatoms), microflagellates, zooplankton, and nutrients (nitrate, phosphate, and silicate) in bag S2 (duplicate bag with low concentration of sediment) expressed as a ratio relative to concentrations in control bag C1.

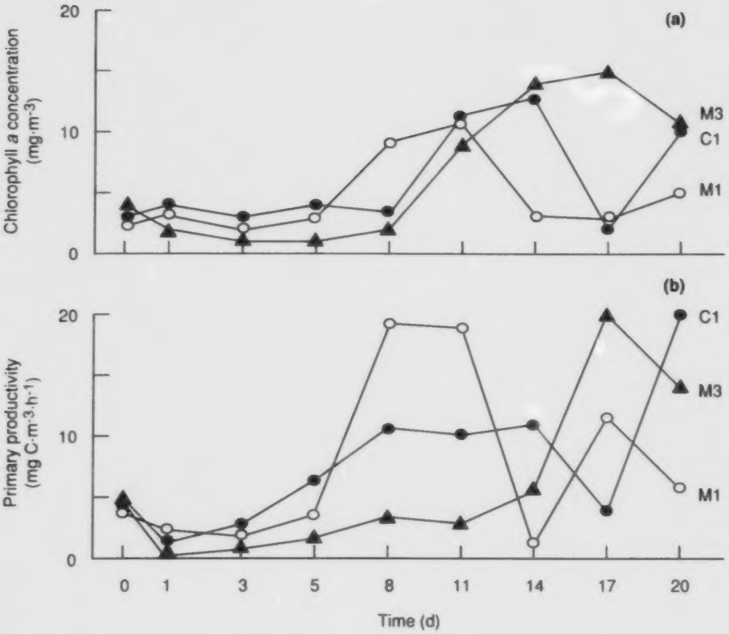

Fig. 5. Changes in chlorophyll *a* concentrations and primary productivity over time in the control bag (C1), low-metal bag (M1), and high-metal bag (M3).

that in M1 (less polluted). Phytoplankton abundance in S3 was similar to that in S1 and S2 except that the peak occurred earlier in S3. The fact that the number of phytoplankton was higher in M3 than in M1 and S1 and that the peak occurred earlier appears to be closely related to the amount of zooplankton in M3, which did not increase in the beginning, but decreased until the end of the experiment (see Chen et al. (this volume) for further discussion on zooplankton).

Diatom cell numbers reached their peak at the same time as the numbers of the two herbivores decreased to their lowest value during the entire experiment. Therefore, we conclude that the decrease in the amount of zooplankton reduced grazing pressure; consequently, the number of diatoms increased. This increase in the number of diatoms in M3 over the control is also reflected in the increased chlorophyll *a* concentrations during the last week of the experiment in M3 and the increase in primary productivity on day 17 (Fig. 5).

Phytoplankton species composition

The phytoplankton classes observed during the experiment were *Bacillariophyceae*, *Pyrrophyceae*, *Chrysophyceae*, *Chlorophyceae*, *Euglenophyceae*, *Cryptophyceae*, and *Cyanophyceae*. Planktonic diatoms were the major group,

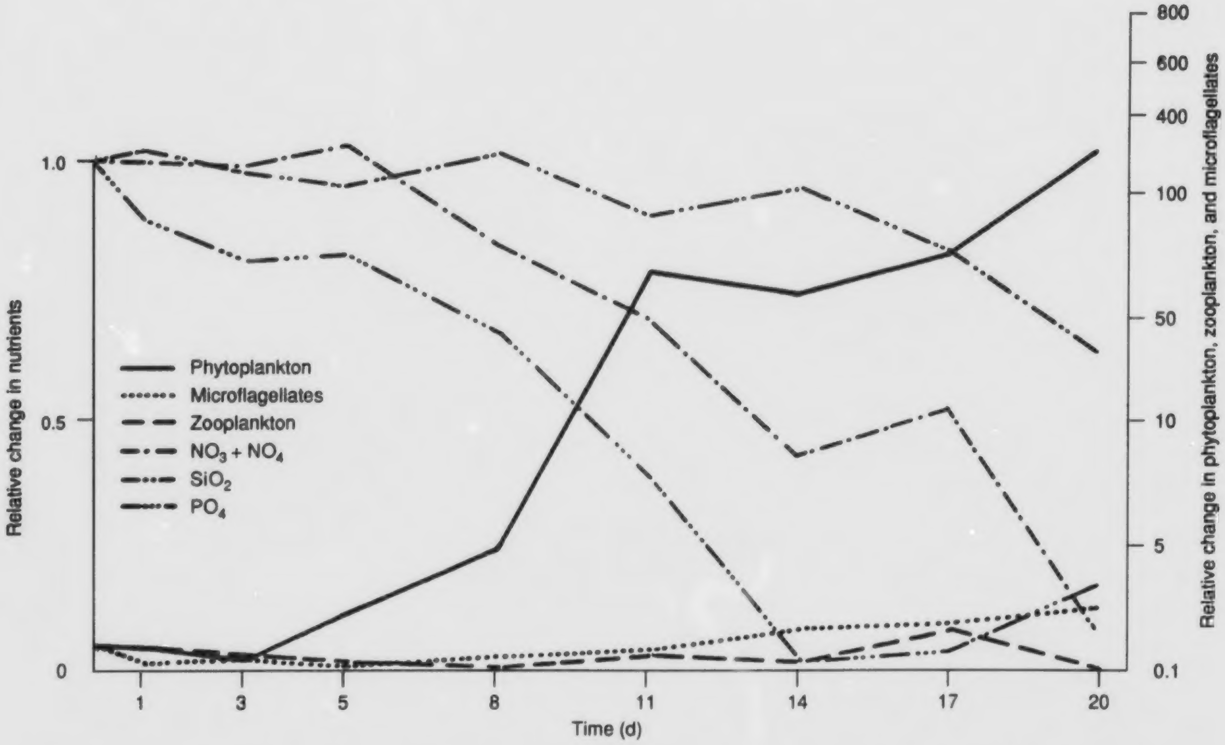

Fig. 6. Changes in phytoplankton (mainly diatoms), microflagelletes, zooplankton, and nutrients (nitrate, phosphate, and silicate) in bag M3 (high concentration of heavy metal mixture) expressed as a ratio relative to concentrations in control bag C1.

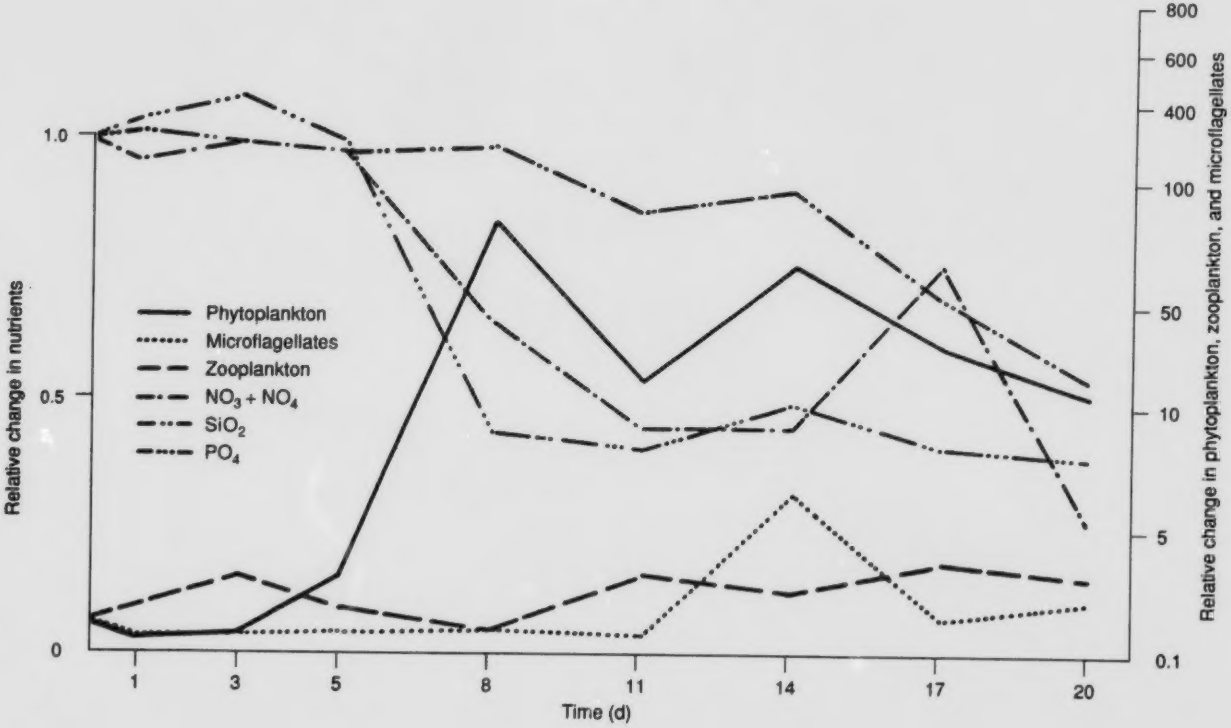

Fig. 7. Changes in phytoplankton (mainly diatoms), microflagelletes, zooplankton, and nutrients (nitrate, phosphate, and silicate) in bag S3 (high concentration of sediment) expressed as a ratio relative to concentrations in control bag C1.

consisting of 25 genera: 10 genera of centric diatoms and 15 genera of pennate diatoms. The dominant diatom species were *Skeletonema costatum* and *Cylindrotheca closterium*. *Chaetoceros* spp and *Cerataulina* were present throughout most of the experiment. Other genera of centric diatoms present in very low numbers were *Corethron*, *Coscinodiscus*, *Eucampia*, *Hemiaulus*, *Rhizosolenia*, and *Schroderella*. Among pennate diatoms, *Nitzschia* spp were the most abundant, followed by *Cylindrotheca closterium*, *Navicula* spp, *Amphora*, and *Amphiprora*. In addition to planktonic diatoms, euglenophytes, dinoflagellates, and chrysophytes were also present in minor proportions. The dominant fraction of the microflagellates were the smallest cells, 1–5 μm.

Centric diatoms were dominant in the control, whereas pennate diatoms were dominant in the polluted bags (Table 3). *Thalassiosira* spp occurred only in the control and the less polluted bags. These results suggest that centric diatoms are more sensitive to the pollutants than pennate diatoms.

Thomas and Seibert (1977), Thomas et al. (1977), and Harrison et al. (1986) also observed that centric diatoms were more sensitive to heavy metals and oil pollution than pennate diatoms. It is interesting to note that the euglenophytes increased only in the bag with the highest concentration of heavy metals (Fig. 8), demonstrating a strong tolerance by this group to heavy metals.

In summary, diatom cell numbers increased in the bags (M1, S1, and S2) with low concentrations of pollutants during the first 3 d of the experiment and increases were faster than that in the control (C1), perhaps due to a reduction in grazing pressure. During the first 3 d, phytoplankton cell numbers decreased in the bags (M3 and S3) with the highest concentration of pollutants, indicating that diatom growth was inhibited by pollution stress.

Table 3. Centric and pennate diatom cell numbers (cells·mL^{-1}) in each bag.

		Day								
Group	Bag	0	1	3	5	8	11	14	17	20
Centric diatoms	C1	17	—	61	150	2 542	11 141	15 968	1 363	6 401
	M1	14	58	79	26	53	18	26	97	105
	M3	22	19	9	149	352	4 783	4 221	5 311	18 641
	S1	—	—	26	44	299	1 442	548	353	—
	S2	7	—	—	141	—	353	484	167	70
	S3	—	—	—	35	2 059	370	1 055	739	141
Pennate diatoms	C1	8	27	9	9	184	308	388	62	70
	M1	10	14	62	18	44	97	115	168	106
	M3	51	53	18	18	—	35	70	106	492
	S1	21	81	26	18	61	133	316	440	105
	S2	14	28	53	18	45	352	1 196	353	667
	S3	27	9	18	44	70	36	634	105	140

Note: C1 = control, M1 and M3 = low and high concentration of metal mixture added, S1 and S2 = duplicates, low sediment addition, S3 = high sediment addition.

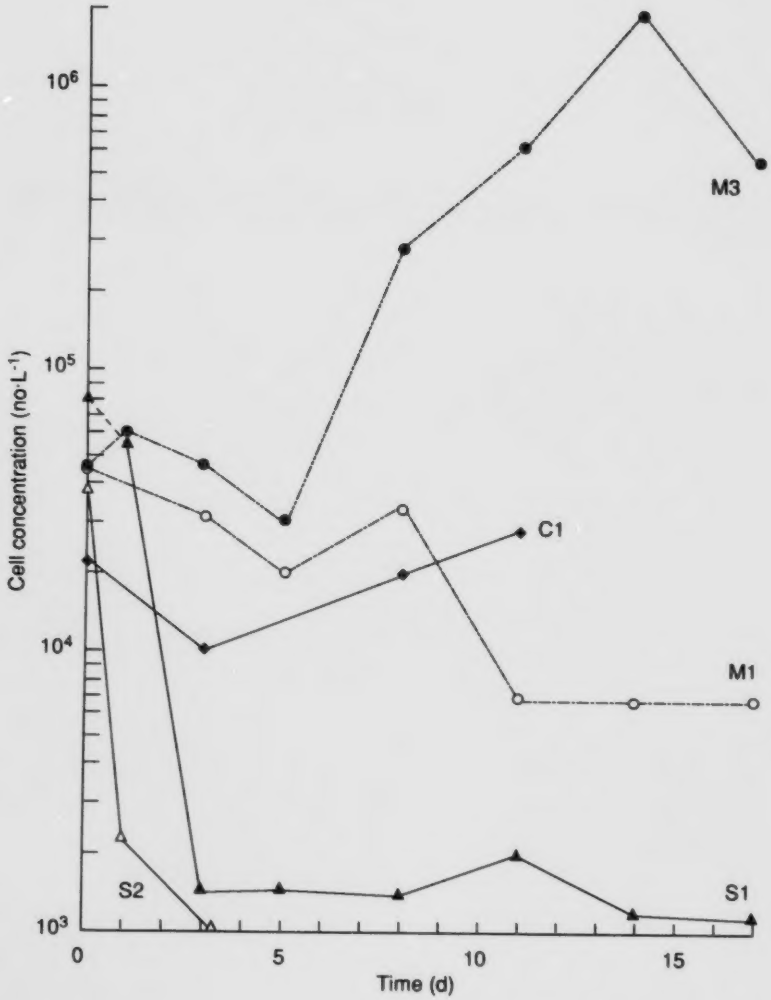

Fig. 8. Change in abundance of euglenophytes in the bags over time.

Changes in cell numbers of planktonic diatoms or microflagellates were also accompanied by increases or decreases in zooplankton because the stress from the added pollutants affected not only planktonic diatoms but also zooplankton. Consumption of nutrients as a result of increases in phytoplankton cell numbers and competition for nutrients between the two groups of phytoplankton, microflagellates and diatoms, affected phytoplankton cell numbers. Future experiments should investigate interactions among pollutants and the effects of pollutants on phytoplankton growth, population structure, and succession.

Acknowledgment

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Harrison, P.J., Cochlan, W.P., Acreman, J.C., Parsons, T.R., Thompson, P.A., Dovey, H.M., Chen, X. 1986. The effects of crude oil and Corexit 9527 on marine phytoplankton in an experiment enclosure. Marine Environmental Research, 18, 93–109.

Hollibaugh, J.T., Seibert, D.L.R., Thomas, W.H. 1980. A comparison of acute toxicity of ten heavy metals to phytoplankton from Saanich Inlet, BC, Canada. Estuarine and Coastal Marine Science, 10, 93–105.

Parsons, T.R., Maita, Y., Lalli, C.M. 1984. A manual of chemical and biological methods of seawater analysis. Pergamon Press, New York, NY, USA. 173 pp.

Thomas, W.H., Seibert, D.L.R. 1977. Effects of copper on the dominance and the diversity of algae: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 23–33.

Thomas, W.H., Holm-Hansen, O., Seibert, D.L.R., Azam, F., Hodson, R., Takahashi, M. 1977. Effects of copper on standing crop and productivity: controlled ecosystem pollution experiments. Bulletin of Marine Science, 27, 34–43.

Thomas, W.H., Hollibaugh, J.T., Seibert, D.L.R., Wallace, G.T., Jr. 1980. Toxicity of a mixture of ten metals to phytoplankton. Marine Ecology Progress Series, 2, 213–220.

A Gray Model for Studying the Effects of Metals on Phytoplankton Growth in Marine Ecosystem Enclosed Experiments

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Data from an experiment investigating the effect of heavy metals (Cd, Cu, Pb, Zn, and Hg) on phytoplankton growth in marine enclosed ecosystems were analyzed statistically. The experiment was carried out from 18 April to 8 May 1985 in Xiamen, People's Republic of China, with the cooperation of Canadian scientists. A nonsteady-state growth model for phytoplankton in this disturbed and dynamic ecosystem is proposed based on the Michaelis–Menten principle of enzyme kinetics. Parameters in the growth equation were calibrated using data derived from experimental observations. Growth patterns of phytoplankton exposed to two concentrations of heavy metals were discussed with the calibration results.

Use of Enclosed Experimental Ecosystems to Study the Effects of Suspended Sediments on Zooplankton Communities

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Large volumes of natural seawater were enclosed in plastic bags for experimental ecosystem studies in both Xiamen, People's Republic of China, and Saanich Inlet, BC, Canada. At both sites, various concentrations of sediment were added to the experimental containers and changes in resident zooplankton communities were followed. In the Canadian experiment, the added sediment was composed of fine-grained mine tailings that are routinely discharged into the sea from a copper mine. In the People's Republic of China experiment, the added sediment was obtained from dredgings in the discharge area of a Xiamen fertilizer factory. The effects of both sediment types on zooplankton species composition and abundance were compared, paying particular attention to differential effects on larvaceans (appendicularians), which feed on small particle sizes, versus herbivorous copepods, which consume larger food items. Mine tailings at concentrations of 40 and 300 ppm increased total numbers of both larvaceans and copepods over those in the control, possibly by both delaying and increasing primary productivity. On the other hand, dredged polluted sediments at concentrations of 11.2 and 112 ppm appreciably depressed total numbers of copepods compared with controls, but did not produce a consistent pattern with respect to Oikopleura *numbers.*

Enclosed experimental ecosystems have been used to study the effects of various types of suspended sediments on planktonic communities. This paper focuses on the responses of and changes in resident zooplankton in containers when they are exposed to mine tailings that are routinely discharged into the sea from a Canadian copper mine and to sediment obtained from the discharge area of a fertilizer factory in Xiamen, People's Republic of China.

Methods

The first experiment was conducted in three 60-t controlled experimental ecosystems (CEEs) deployed in Saanich Inlet, BC, Canada, as part of a joint research project between the People's Republic of China and Canada. One container

(CEE-1) was a control; CEE-2 and CEE-3 were treated with dredged mine tailings at concentrations of about 40 and 300 ppm.

The second experiment was conducted in Xiamen in 1985. Three enclosures containing about 10 m^3 of seawater were employed as experimental systems and received sediment dredged from the discharge area of a Xiamen fertilizer factory. Containers S1 and S2 received about 11.2 ppm and S3 about 112 ppm of the polluted sediment.

In both experiments, zooplankton in control and experimental containers were routinely sampled using a 20-cm diameter net with 200-µm mesh. The net was towed from near the bottom of each container to the surface (13 m to surface in the Saanich Inlet experiment; 3 m to surface in the Xiamen experiment).

Results and discussion

Species composition of the zooplankton communities in both experiments (Table 1) was remarkably similar given the differences in latitude (ca. 48° N versus 24° N). The dominant herbivorous copepods in both areas were *Paracalanus parvus* and species of *Acartia* (*A. clausi* in Saanich Inlet and *A. pacifica* in Xiamen). *Pseudocalanus minutus* was also an important member of the herbivorous zooplankton community in the Saanich Inlet experiment. The cyclopoid copepod *Oithona similis* was present in both areas, but was much more abundant in Saanich Inlet waters; it is considered to be primarily herbivorous in this study, although it is capable of feeding on a wide size spectrum, which may include smaller zooplankton (e.g., Poulet 1978). Both experiments also included small numbers of the predatory copepod *Corycaeus*, but a major difference involved the presence of a large predator, *Tortanus derjuginii*, in the Xiamen containers. The food habits of *Tortanus* in Xiamen waters have been discussed by Lee (1964); this copepod fed on *Acartia pacifica* in laboratory investigations conducted as part of the present study. Larvaceans (mostly species of *Oikopleura*) were also important zooplankton in both experiments.

Table 1. Comparison of dominant zooplankton species in enclosed experimental ecosystem experiments conducted in Saanich Inlet, BC, Canada, and Xiamen, People's Republic of China.

Saanich Inlet	Xiamen
Copepoda	Copepoda
Paracalanus parvus	*Paracalanus parvus*
Pseudocalanus minutus	
Acartia clausi	*Acartia pacifica*
Oithona similis	*Oithona similis*
Corycaeus anglicus	*Corycaeus affinis*
	Tortanus derjuginii
Larvacea	Larvacea
Oikopleura dioica	*Oikopleura* sp.
Fritillaria borealis	

Note: Data supplied by C. Lalli, Lin Jinmei, and Chen Xiaolin.

There was also considerable similarity in the numbers of zooplankton and in changes in their population densities in control containers in both the Saanich Inlet and Xiamen experiments (compare controls in Figs 1 and 2). Copepods attained maximal numbers late in the experiments, reaching densities of about 2 000 individuals·L^{-1} in Saanich Inlet and about 3 000 individuals·L^{-1} in Xiamen controls. In both cases, maximal numbers of larvacea in controls were attained in less than a week and differed by less than 100 individuals·L^{-1} (i.e., 378 individuals·L^{-1} in Saanich Inlet versus 473 individuals·L^{-1} in Xiamen).

The addition of mine tailings increased maximal numbers of both larvaceans and herbivorous copepods in each of the experimental containers compared with the control during the 3-week posttreatment period (Fig. l). *Corycaeus*, a predatory copepod, followed the same response curve as the herbivorous copepod species, increasing in numbers in both experimental containers compared with the control. This enhancement of zooplankton densities may be attributed to a delay in the onset of primary production caused by decreases in the depth of the euphotic zone and to a subsequent increase in total primary production that resulted in better coupling between phytoplankton and zooplankton (Parsons et al. 1986). There was a clear separation between peak numbers of larvaceans, which appeared earlier in the experiment, and maximal numbers of copepods, which developed later. This may have been a reflection of differences in feeding habits — larvaceans feed on small-sized particles, such as bacteria and microflagellates, whereas copepods prefer larger food items. This temporal separation may also have resulted from the faster generation times of larvaceans versus copepods.

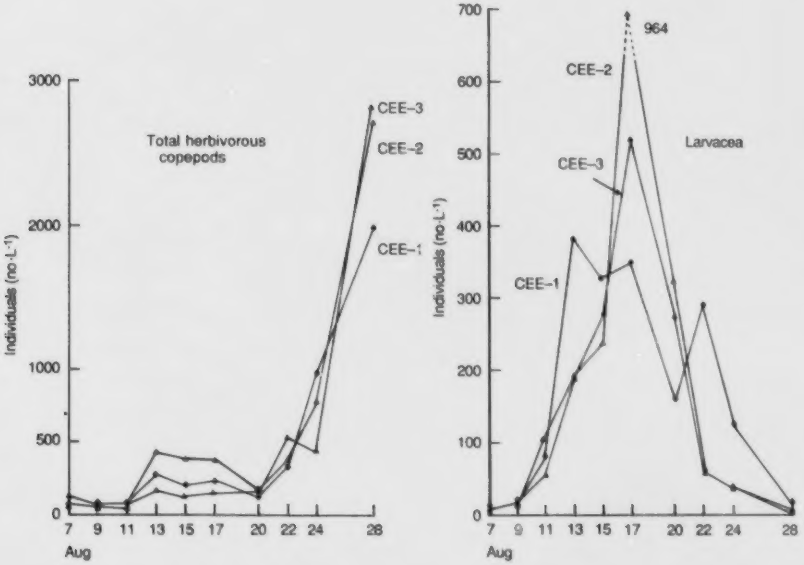

Fig. 1. Zooplankton densities in enclosed marine ecosystem experiments conducted in Saanich Inlet, BC, Canada, during 1984. CEE-1 = control; CEE-2 = 40 ppm mine tailings; and CEE-3 = 300 ppm mine tailings.

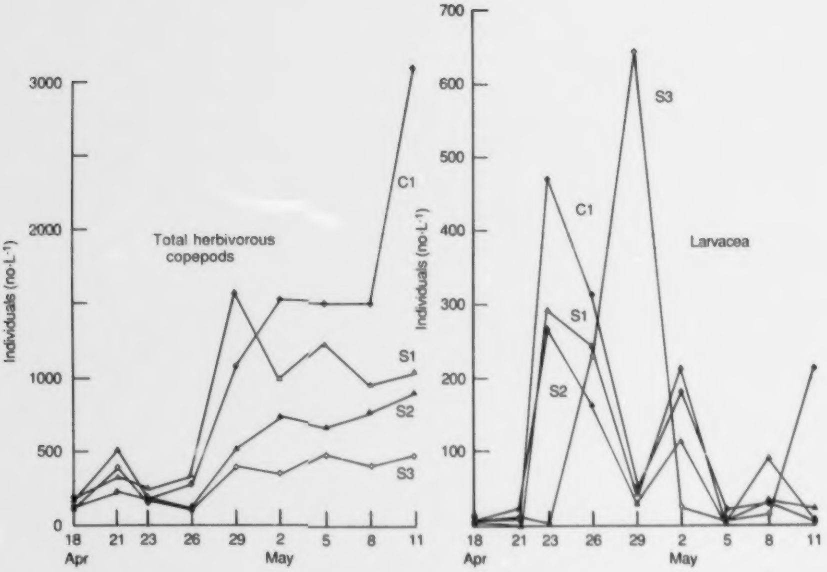

Fig. 2. Zooplankton densities in enclosed marine ecosystem experiments conducted in Xiamen, People's Republic of China, during 1985. (Data supplied by C. Lalli, Lin Jinmei, and Chen Xiaolin.) C1 = control; S1 = 11.2 ppm polluted sediment; S2 = 11.2 ppm polluted sediment; and S3 = 112 ppm polluted sediment.

In the Xiamen experiment, additions of polluted sediment did not enhance zooplankton numbers. The highest levels of sediment (112 ppm in S3) appreciably depressed numbers of total copepods; this effect was particularly noticeable after 29 April (day 11), when maximal numbers were consistently present in the control (C1) and lowest in S3. The two containers with lower levels of sediment (11.2 ppm) developed copepod densities that were intermediate between those of the control and S3. The effects of suspended polluted sediment on larvacea were not as clear. The development and decline of populations in S1 and S2 were similar to the control pattern, but peak numbers of larvaceans were somewhat lower in these experimental enclosures. The highest sediment levels delayed the bloom of larvaceans in S3 by about 6 d, a result comparable to that obtained in experiments using suspended mine tailings. Carnivorous copepods (*Tortanus* and *Corycaeus*) reached maximal densities during the first 3 d (highest value = 94 individuals$\cdot L^{-1}$), then they declined rapidly and generally remained below 30 individuals$\cdot L^{-1}$ in all bags.

Conclusions

Changes in zooplankton densities or species composition, or both, can be used as indicators of environmental changes. It is important to stress that only dominant species need to be identified in the communities and that knowledge of their general biology is as important as taxonomic identification. Separation of zooplankton into trophic groups (e.g., herbivores versus carnivores; bacterial/flagellate feeders versus diatom grazers) allows one to analyze more accurately the changes in population densities of particular species or groups and to link these with changes in microbial and phytoplankton ecology. Similarly, separation of groups with different generation times (e.g., larvacea versus copepods) permits better analysis of population dynamics. Total zooplankton numbers mask simultaneous declines and increases of two or more groups and will usually not provide much information on biological or ecological changes occurring in either enclosed experimental ecosystems or in natural environments.

Lee, S-C. 1964. Preliminary studies on the food and feeding habits of some marine planktonic copepods in Amoy waters. Journal of Xiamen University, 11, 93–109.

Parsons, T.R., Thompson, P., Wu, Y., Lalli, C., Hou, S., Xu, H. 1986. The effect of mine tailings on the production of plankton. Acta Oceanologica Sinica, 5, 417–423.

Poulet, S.A. 1978. Comparison between five coexisting species of marine copepods feeding on naturally occurring particulate matter. Limnology and Oceanography, 23, 1 126–1 143.

Effects of Heavy Metals and Sediment on Zooplankton in Marine Ecosystem Enclosed Experiments

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*Eight experimental ecosystem enclosures were employed to test the ecological effects of pollutants on marine zooplankton. Results indicated that low levels of mixed metals, including Cd, Cu, Hg, Pb, and Zn, inhibited growth and development of herbivorous copepods; this inhibition was stronger with higher concentrations of metals. Low levels of polluted sediment obtained from dredgings near a fertilizer factory also inhibited development of populations of herbivorous copepods, the inhibition being more pronounced with higher concentrations of sediment. Carnivorous copepods did not show obvious responses to either metals or sediment. Larvaceans (*Oikopleura *sp.) exhibited an obvious response to both low and high levels of metals, but not to sediment additions.*

In recent years, a number of studies have reported on the results of adding a single heavy metal, mainly Cd, Cu, or Hg, to marine experimental ecosystems to investigate the effects of these metals on zooplankton, such as copepods, ctenophores, and larvaceans (Beers et al. 1977; Gibson and Grice 1977; Reeve et al. 1977; Kuiper 1982). During 1985, Chinese and Canadian scientists working in Xiamen, People's Republic of China, designed a marine ecosystem experiment in which a mixture of Cd, Cu, Hg, Pb, and Zn was added to some enclosures and sediment from a waste-dumping site was added to other experimental enclosures. It was felt that the experimental design would closely simulate actual conditions of marine heavy-metal pollution, and that the results would be important in understanding ecosystem processes and changes resulting from pollution stress, assessing aquatic environmental quality, and establishing environmental-protection policy.

This paper reports on the effects of heavy-metal and polluted-sediment additions on zooplankton community structure in experimental enclosures.

Methods

The eight experimental enclosures consisted of bags suspended in a tank containing seawater. Bags C1 and C2 served as controls. Bags M1 and M2 received a mixture of heavy metals at concentrations of 1.0 ppb Cd, 3.5 ppb Cu, 0.2 ppb Hg, 0.3 ppb Pb, and 3.5 ppb Zn. Bag M3 received higher concentrations of metals (5.0 ppb Cd, 10.0 ppb Cu, 1.0 ppb Hg, 1.5 ppb Pb, and 17.5 ppb Zn). Low concentrations (11.2 ppm) of sediment dredged from near a fertilizer factory were added to bags S1 and S2, whereas bag S3 received 112 ppm of this sediment. Additional details on the experimental design are contained in a general data report (unpublished report titled: *MEEE–85–Xiamen: the addition of trace metals and sediments to controlled experimental ecosystems in Xiamen. MEEE experiment in the Third Institute in Xiamen, April 18–May 15, 1985*).

A total of 72 zooplankton samples were collected using a 202-µm pore-size plankton net measuring 2.5 cm in diameter and 70 cm in length. The net was towed from 3 m to the surface, through the centre of the enclosures, between the hours of 0800 and 0900. Each sample was concentrated to a volume of 96 mL, which was then preserved with 4 mL of formalin. All zooplankton in the samples were placed in Bogorov counting chambers and identified and counted under a dissecting microscope. Abundance was expressed as the number of individuals per cubic metre of seawater, assuming that each plankton tow filtered 0.11 m^3.

Copepods were the dominant group of zooplankton in all bags, and they were classified according to trophic level. *Acartia pacifica*, *Paracalanus parvus*, *P. aculeatus*, *Oithona similis*, *Labidocera euchaeta*, and *Pseudodiaptomus marinus* were classified as herbivorous species, whereas *Tortanus derjuginii* and *Corycaeus affinis* were identified as carnivores. Unidentified copepod larvae and nauplii were not included in these two groupings; they constituted less than 5% of the total copepods. Larvaceans were sometimes abundant in the samples, but they were often damaged during sampling. In such cases, detached heads were counted, but not detached tails, to avoid overrepresentation.

Results

For convenience, the experimental period has been divided into three stages: the early stage, 18–26 April; the middle stage, 26 April–5 May; and the late stage, 5–11 May.

Control bags

Only data from bag C1 are included here as bag C2 was damaged during the experiment. As shown in Fig. 1, herbivorous copepod numbers remained relatively stable during the early stage, but increased dramatically during the middle stage reaching a plateau on 2 May at which the numbers (15 230 individuals·m^{-3}) were about 13 times the initial density. The population stabilized at this density until 8 May, when numbers rose rapidly to reach 31 140 individuals·m^{-3} (26 times the initial density) on 11 May, at the end of the experiment. *Paracalanus parvus* and *Acartia pacifica* were the dominant species, constituting 99.1% of the total

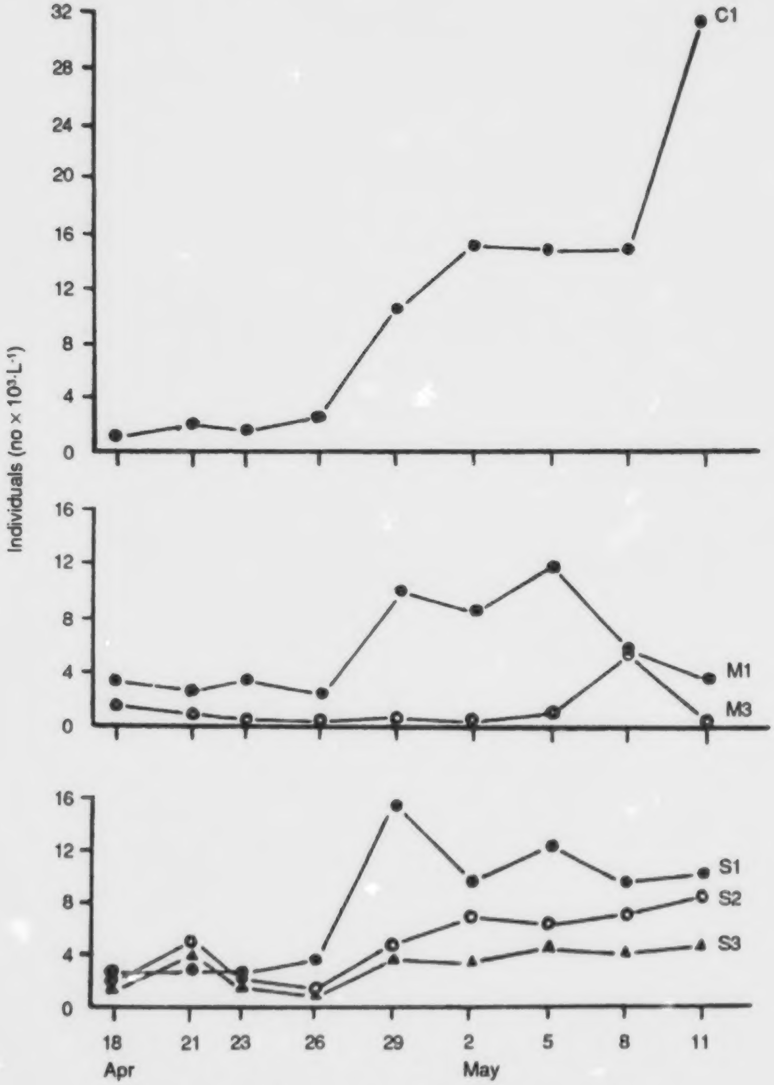

Fig. 1. Changes in the numbers of herbivorous copepods.

herbivorous copepods on 2 May, and 99.4% on 11 May. Numbers of carnivorous copepods remained low throughout the experiment, varying between 40 and 520 individuals·m^{-3}. Larvacea were relatively abundant during the middle stage, ranging between 510 and 4 730 individuals·m^{-3}. *Pleurobrachia globosa* was present only occasionally during the middle and late periods, at densities of 10–20 individuals·m^{-3}. There were only a few other zooplankton groups, such as meroplanktonic larvae, all of which were present in low numbers only.

Heavy-metal bags

Only data from bag Ml were used as there were technical problems with duplicate bag M2. The density of herbivorous copepods (Fig. 1) was low and relatively stable during the early stage, and was similar to the control bag in this respect. Numbers rose to a small peak on 29 April, and the density remained at about the same level for the next 6 d. Numbers reached their maximum density on 5 May (12 190 individuals·m^{-3}) at four times the initial density. Numbers decreased gradually during the late stage to a level similar to the initial population density. Species composition and relative abundance of zooplankton were similar to those of the control, but numbers of larvacea were much lower during the middle stage compared with C1.

Results obtained from bag M3, to which higher concentrations of heavy metals were added, were quite different. During the early stage, numbers of herbivorous copepods decreased gradually from an initial density of 1 700 individuals·m^{-3} to 250 individuals·m^{-3} (Fig. 1). Numbers remained low until 8 May, when they reached 5 390 individuals·m^{-3}, after which the density declined to very low values by the end of the experiment. *Paracalanus parvus* was the dominant species, representing 97% of the total herbivorous species on 8 May. Numbers of carnivorous copepods and changes in species composition were similar to those observed in bag M1.

Sediment bags

Numbers of herbivorous copepods in bag S1 increased very slightly during the early stage, but increased rapidly after 26 April (Fig. 1). By 29 April, the density reached 15 720 individuals·m^{-3}, or eight times the initial density. Thereafter, numbers decreased gradually, but remained above 10 000 individuals·m^{-3} at the end of the experiment. Numbers of herbivorous copepods in bag S2, a duplicate of Sl, were generally lower throughout the experiment. After a slight decline during the early stage, numbers gradually increased to the end of the experiment, reaching a density of 8 980 individuals·m^{-3}, or five times the initial density. In bag S1, *Pleurobrachia* was present at a density of 250 individuals·m^{-3} at the end of the experiment.

During the early stage, herbivorous copepods in bag S3, containing a higher level of sediment, were present in numbers similar to those in bag S2. As well, herbivorous copepods in bag S3 underwent similar density changes as those in bag S2. Numbers increased from 26 April to the end of the experiment, but the density was always lower than that in bags S1 and S2. The highest density (4 850 individuals·m^{-3}) was attained during the late stage and was only 3.5 times the initial density. Carnivorous copepods and other zooplankton in S3, as in S1 and S2, had a species composition and relative abundance similar to those of the control.

Discussion and conclusions

The zooplankton population in the control bag followed a normal growth curve, which tracked the growth of phytoplankton (Fig. 2). Additions of either the mixture

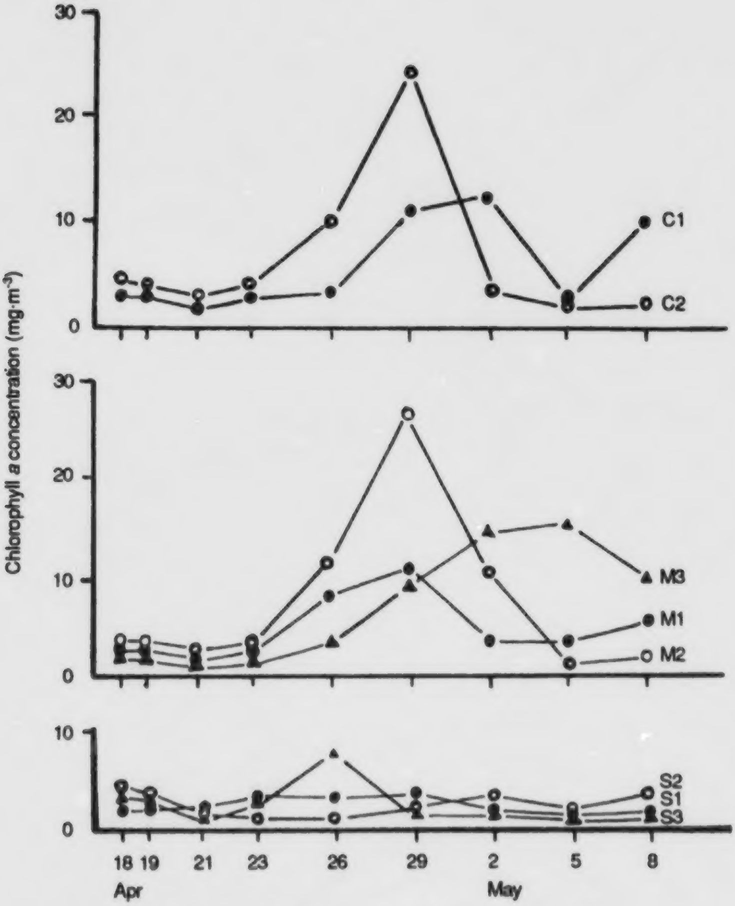

Fig. 2. Changes in chlorophyll *a* concentrations with time.

of heavy metals or the polluted sediment altered zooplankton populations in the experimental bags.

A low concentration of mixed heavy metals depressed the growth and development of herbivorous copepods (bag Ml) to some degree. The highest density in bag M1 during the experiment was only 33% of that of the control. When the concentration of mixed heavy metals was increased, inhibition increased as well, with the maximal density attained in bag M3 being only 17% of that of the control. The chlorophyll *a* concentration (Fig. 2) indicated that phytoplankton in Ml were not affected by the addition of low concentrations of heavy metals, but were depressed by the higher concentrations added to bag M3. Therefore, inhibition of copepod population growth in Ml may have resulted from direct toxic effects of the pollutants, whereas herbivorous copepods in M3 may have also been affected by depression of their food supply (Qian et al., this volume). Mixed heavy metals at both low and high concentrations severely depressed growth and development of larvacean populations.

When a low concentration of sediment (11.2 ppm) was added to the enclosures, it also depressed development of herbivorous copepod populations; maximal density (bags SI and S2) reached only 50% of that of the control. When the concentration was increased to 112 ppm, inhibition was more apparent and the maximal density (in bag S3) was lowered to 16% of that of the control. As shown in Fig. 2, phytoplankton development was very much reduced in all bags to which sediment was added, probably because of light limitations. The low food supply for zooplankton must have inhibited development of the herbivorous copepod population. On the other hand, growth and development of larvaceans, which feed on bacteria and nanoplankton, were normal during the experiment. These observations suggest that direct toxic effects of the sediment were minimal, unlike those produced by heavy metals.

Acknowledgments

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Beers, J.R., Stewart, G.J., Hoskins, K.L. 1977. Dynamics of micro-zooplankton populations treated with copper: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 66–79.

Gibson, V.R., Grice, G.D. 1977. Response of macro-zooplankton populations to copper: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 85–91.

Kuiper, J. 1982. Ecotoxicological experiments with marine plankton communities in plastic bags. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 181–193.

Reeve, M.R., Gamble, J.C., Walter, M.A. 1977. Experimental observations on the effects of copper on copepods and other zooplankton: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 92–104.

Relationships Between Particle Characteristics and Biological Activities in Controlled Ecosystems

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Relationships between particle characteristics and biological activities were studied at the Marine Ecosystems Research Laboratory, University of Rhode Island, USA, in 1983 and at the Third Institute of Oceanography, Xiamen, People's Republic of China. Eutrophication was found to alter the structure and dynamics of the entire ecosystem; consequently, the composition and properties of particles in the system were changed. Benthic filter feeding was also found to enhance removal rates of particles and their associated elements. These results indicated the importance of the sediment–water interface of a shallow aquatic ecosystem. As the suspended particles sank and were deposited as sediment, particulate elements became progressively enriched and strongly bound to particles due to diagenetic processes. Because organisms dominated the particulate flux in the system, the C:N ratio of the particle was similar to the Redfield ratio. In addition to chemical analysis, scanning electron microscopy was also used to observe the nature of particles. In summary, marine mesocosms provide an useful tool to study the effects of biological activities on the dynamics of particulate matter.

Because of its compositional complexity and wide-ranging chemical activity, particulate matter in the ocean plays an important role in transporting elements in and removing elements from seawater. The binding and release of particulate elements depend not only on the total amount of particles but also, and most importantly, on the characteristics of the particles. These characteristics, which include composition, size, surface properties, structure, etc., depend on the source of the particles and various changes in physical, chemical, and biological processes occurring in the marine environment. Conversely, changes in these characteristics also affect the role of particulate matter on biogeochemical cycling of elements and transferring polluants in and removing pollutants from the ocean. Previous studies have often neglected the effect of dynamic changes, especially the effects of biological processes, on the characteristics of particulate matter.

Biological activities affect the characteristics of particles both directly and indirectly. Living organisms, as a component of particulate matter, can actively absorb or passively adsorb various nutrients, trace elements, or toxic elements. During subsequent metabolism, these elements can again be released and recycled in the water, or they can be transported to greater depths in the ocean by sinking with organic matter or by passing through the food chain. Organisms acting indirectly through their metabolic or decomposed products can also alter the surface chemistry of inorganic particles, causing them to behave like organic particles.

This paper summarizes the results of two studies on marine particulates — one conducted at the Marine Ecosystems Research Laboratory (MERL) at the University of Rhode Island, USA, in 1983 and the other carried out at the Third Institute of Oceanography, Xiamen, People's Republic of China, in 1985. These results are used to elucidate the relationship between the characteristics of particulate matter and biological activities in the ocean.

Materials

The MERL project was an experiment on eutrophication and Cu toxicity of a marine ecosystem (Nixon et al. 1984). At the beginning of the experiment, a given composition of natural seawater, bottom sediment, and biological populations from the bay were transferred to a control tank and a eutrophication tank. To simulate the residence time of seawater and tidal action in the bay, the water in each tank was renewed at a rate of 4% per day and was also stirred for 2 h every 4 h.

Suspended particles, sinking particles, and bottom sediment were also collected for analysis. Suspended particles were obtained by centrifugation. Sinking particles were collected for 24 h using two sediment traps (5.1 cm in diameter by 12.8 cm high) hanging 1 m below the water surface and 0.5 m from the bottom of the tank (Fig. 1). Particulate materials obtained were separated and extracted chemically using a four-step procedure, and were then analyzed for carbon and hydrogen. A scanning electron microscope (SEM) was also used to observe the particles. The results were used to show the effects of eutrophication and biological metabolism on the composition and ultrastructure of particulate matter and the binding efficiency of metals on particles.

During the 1985 Marine Ecosystem Enclosure Experiment (MEEE) on heavy metal pollution (MEEE Group, this volume), samples were collected from the control bag, the bag treated with sediment, and the bag treated with heavy metals (Table 1). Suspended particles were collected by filtering water samples through 0.4-μm Nuclepore membrane filters. Sinking particles were collected using a sediment trap, whereas sediment samples were pumped from the bottom of the bags (Fig. 2). The present study focused mainly on particulate fluxes, carbon and nitrogen composition, the ultrastructure of particles, and variations in the concentration of some major heavy metals due to biological activities.

Table 1 lists some of the experimental conditions in the MERL tanks and MEEE bags. In the MERL experiment, all of the tanks were treated with the same amount of Cu, but with various concentrations of nutrients. In the MEEE, on the other hand, each bag was treated with a different concentration of pollutant, but nutrients were maintained at the same level.

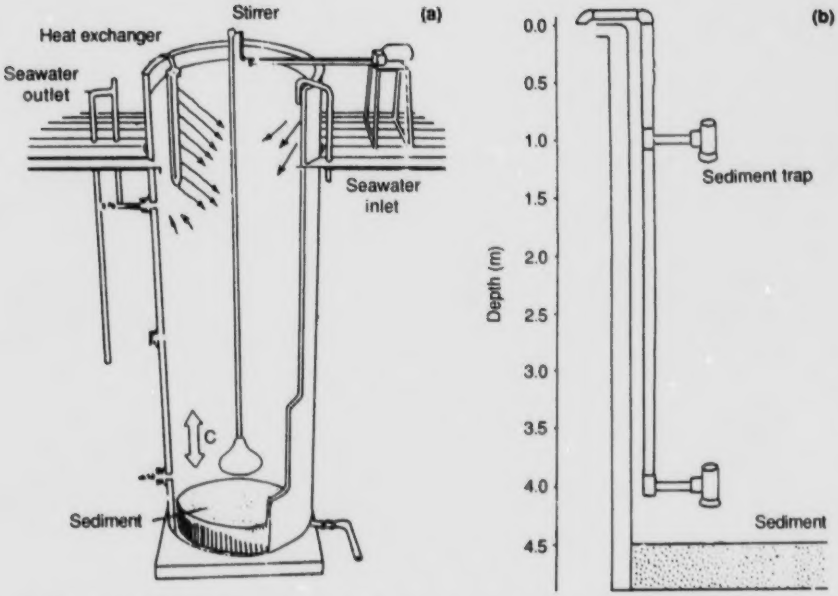

Stirrer
(a)
Heat exchanger
Seawater outlet
Seawater inlet
C
Sediment
(b)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Depth (m)
Sediment trap
Sediment

Fig. 1. (a) MERL experimental tank. (b) Sediment traps used for collecting settling particles.

Table 1. Experimental conditions in MERL tanks and MEEE bags.

Tank/bag	Nutrients	Treatment	Bottom sediment
		MERL (August 1983)	
Control	21:1.6:46.1	Cu (40 ppb)	Yes
Eutrophication	32.7:34.2:65.3	Cu (40 ppb)	Yes
		MEEE (April 1985)	
Control (C1)	5:5:50	None	No
Sediment (S1)	5:5:50	Polluted sediment (11.2 ppm)	
Metals (M1)	5:5:50	Cd (1.0 ppb), Cu (3.5 ppb), Hg (0.2 ppb), Pb (0.3 ppb), Zn (3.5 ppb)	No

Method

Four-step continuous chemical extraction

Each extracted solution was analyzed for Al, Cu, Fe, and Mn, concentrations using an atomic absorptiometer (Perkin-Elmer 5000) by a four-step continuous chemical extraction (Fig. 3). The measurement precision was ± 5–10%.

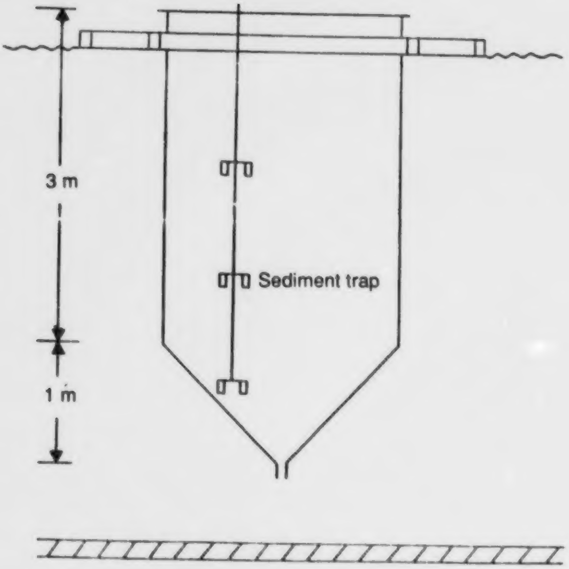
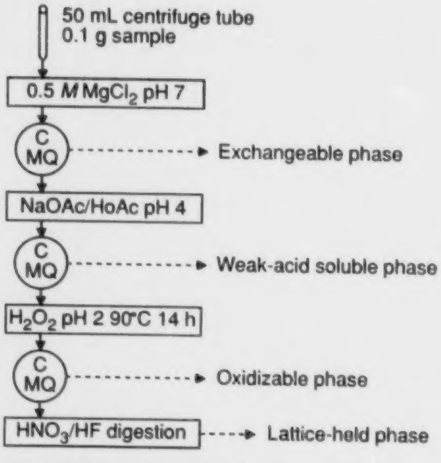

3 m
1 m
Sediment trap

Fig. 2. Schematic of MEEE bag.


50 mL centrifuge tube
0.1 g sample
0.5 M MgCl2 pH 7
C
MQ
Exchangeable phase
NaOAc/HoAc pH 4
C
MQ
Weak-acid soluble phase
H2O2 pH 2 90°C 14 h
C
MQ
Oxidizable phase
HNO3/HF digestion
Lattice-held phase

C: 12 000 g centrifugation for 30 min; MQ rinse

Fig. 3. Flowchart of the four-step continuous chemical extraction method.

Particulate carbon and nitrogen

Suspended particles were collected on a glassfibre filter (Whatman GF/C) that had been preheated at 450°C and preweighed before filtration. The filter and its content were dried and weighed in a low-temperature vacuum oven. The same method was used to obtain the dried weight of sinking particles and sediment. Subsequently, an elemental analyzer (Perkin-Elmer 240 or Carlo-Erba 1106) was used to determine the concentrations of carbon and nitrogen in the sample. Acetanilide was used as a standard and the measurement precision was ±0.1 $mg \cdot g^{-1}$.

Scanning electron microscopy

Particulate matter was spread out on a membrane filter (Nuclepore 0.4-μm), washed thoroughly with distilled water, and air dried. It was then cut and mounted on an aluminum stub and magnified 2 000–10 000 times under a scanning electron microscope (Hitachi S-520) at 15 kV for observing and photographing.

Total concentration

A 50-mg aliquot of dried sample was placed in a Teflon bomb together with a mixture of $HF:HNO_3:HClO_3$ at a ratio of 1:1:0.5. The sample was digested at 120°C for 4 h and then separated by centrifugation. Concentrations of Al, Fe, Mn, Si, Ti, and V were determined using a plasma emission spectrophotometer (WPE-G model).

Results and discussion

Effects of eutrophication

One year after the MERL eutrophication experiment, there were differences in the biomass as well as the community structure of the ecosystems in the two tanks (Table 2). In particular, dominant benthic species, originally composed of bivalve mollusks, were replaced by polychaetes. The amount of suspended matter in the two tanks was the same, but observations under the SEM revealed that the two samples were composed of completely different particles. In the control tank, suspended materials were mainly composed of detritus and inorganic substances, with only small numbers of live organisms and bodies of dead organisms. In the eutrophication tank, on the other hand, suspended materials were mainly composed of various species of diatoms, most of which were alive. Differences in the concentrations of carbon and nitrogen and their ratios also supported these observations (Fig. 4).

Table 2. Parameters from MERL control and eutrophication tanks.

	Control tank	Eutrophication tank
Biomass [chlorophyll *a* ($mg \cdot L^{-1}$)]	1–5	40–80
POC ($mg \cdot kg^{-1}$)	0.7	2.3
PON ($mg \cdot kg^{-1}$)	0.1	0.5
Suspended matter ($mg \cdot L^{-1}$)	14	14
Collection rate ($g \cdot m^{-2} \cdot h^{-1}$)	7.4	0.6
Phytoplankton	Diatom	Diatom (smaller)
Zooplankton	*Acartia tonsa*	*Pseudodiaptomus coronatus* Larvae
Benthos	Bivalves	Polychaetes

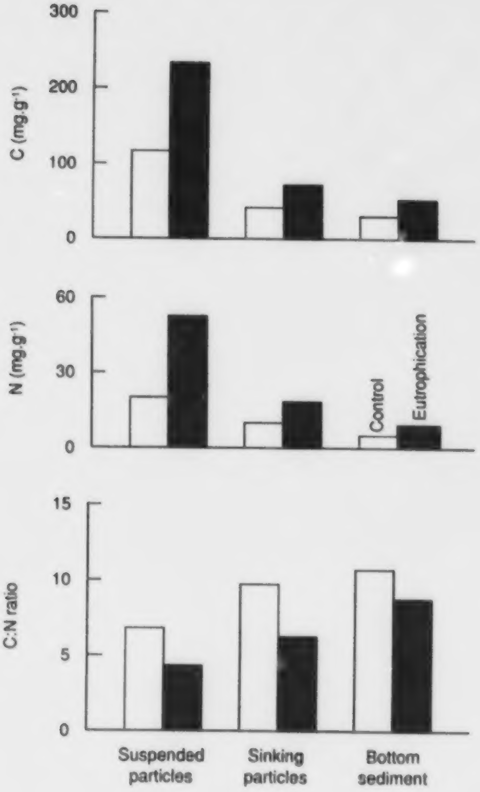


Fig. 4. Concentrations of carbon and nitrogen and C:N ratios in particles from MERL control and eutrophication tanks.

In the eutrophication tank, concentrations of particulate carbon and nitrogen from suspended particles, sinking particles, or bottom sediment were all higher than those in the control tank. The differences were especially large for bottom sediments. There was also a difference in resuspended particles. The collection rate from the lower sediment trap in the control tank was about 10 times the rate observed in the eutrophication tank (Table 2). All of these results indicated that eutrophication not only altered the biomass and the structure of the biotic community but it also altered the composition of the particulate matter in the ecosystem.

As the composition of particles in the control and eutrophication tanks differed, bonding properties between metals and particles also differed. Figure 5 shows the concentrations and percentage of different bonding phases of Fe among the three types of particles found in the two tanks. In terms of the total dried weight of the particles, the amount of Fe in the eutrophication tank was greater than that in the control tank. Percentages of oxidizable and lattice-held Fe were almost identical in suspended particles. For sinking particles, especially those from the upper sediment, the percentage of oxidizable Fe in the eutrophication tank was greater than

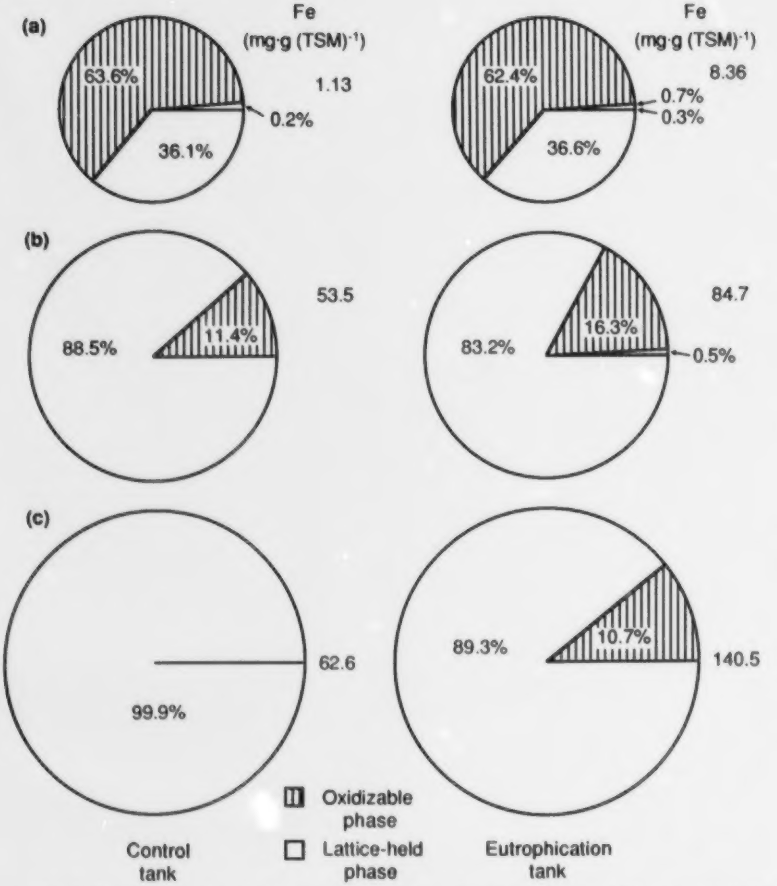


Fig. 5. Total amounts and percentages of various bonding phases of Fe with suspended particles (a), settling particles (b), and bottom sediment (c) in MERL experimental tanks.

that in the control tank; in contrast, the percentage of lattice-held Fe was greater in the control tank. This indicates that as the biotic fraction in particulates increased, the potential for Fe accumulation was elevated and some changes also occurred in bonding properties between Fe and particles. This Fe fraction was easily released to the surrounding solution when dead bodies of organisms or other organic matter were oxidized. Other metals, such as Al, Cu, and Mn, associated with particulates in the two tanks showed similar trends.

Dynamic changes in bonding between metals and particles

Although the depths of the water columns in MERL tanks and MEEE bags were 4.5 and 4 m, respectively, lithification of particles was fairly rapid because particulate matter undergoes biological metabolism or organic oxidation while sinking to

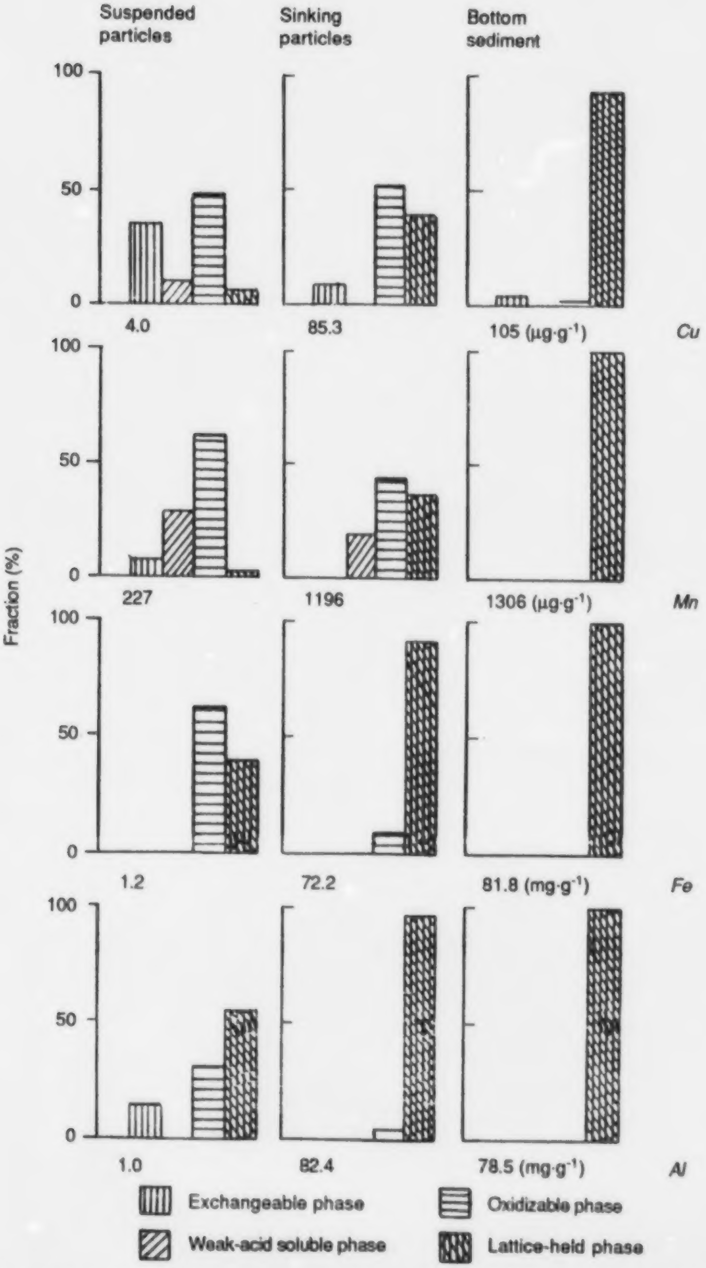


Fig. 6. Variations, according to the sequence suspended particles → settling particles → bottom sediment, in bonding properties of Al, Cu, Fe, and Mn with particulates in MERL experimental tanks.

the bottom. Changes also occurred in both the amount and characteristics of bonding between metals and particles. From suspended particles in the surface water to sinking particles in the water column and finally to bottom sediment, the accumulation of metal onto particles and the bonding strength increased sequentially in this order. The amounts of Al, Cu, Fe, and Mn varied with the types of particle in the control tank (Fig. 6).

Many of these metals showed the most accumulation on bottom sediments, but percentages of various bonding phases differed significantly. For suspended particles, more than 95% Mn, 90% Cu, 60% Fe, and 45% Al existed in the exchangeable, weak-acid soluble, and oxidizable phases. However in bottom sediment, more than 95% of the Cu and nearly 100% of the Al, Fe, and Mn were in the

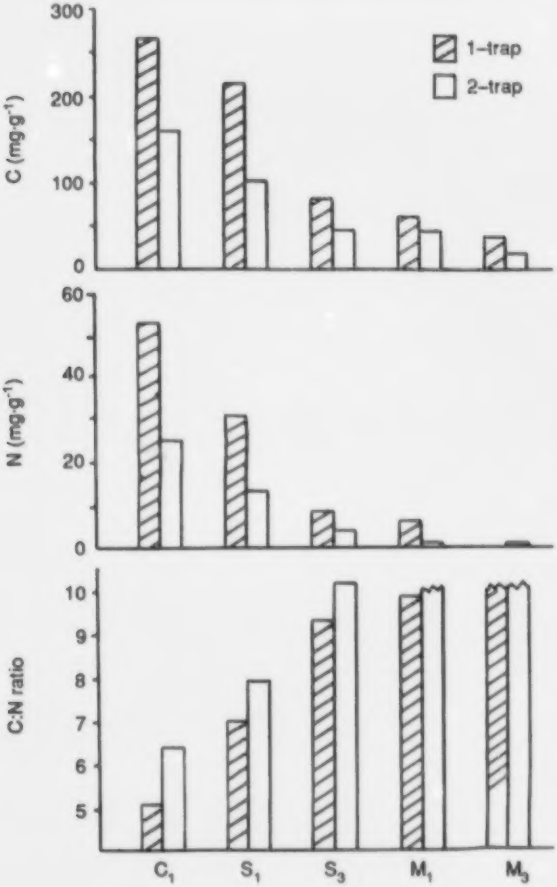


Fig. 7. Concentrations of particulate organic carbon (POC) and particulate organic nitrogen (PON) and C:N ratios of settling particles in MEEE bags on day 8 of the experiment.

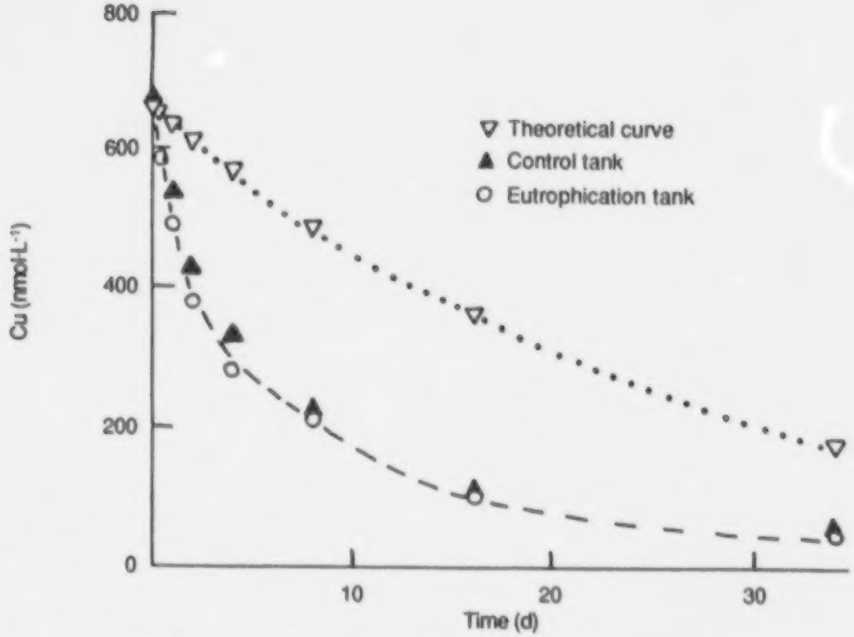


Fig. 8. Transfer curves of externally added Cu in MERL tanks

lattice-held phase. For sinking particles, the bonding strength between metals and particles was in between, although in the case of Fe and Al, it was closer to that of bottom sediment.

Beginning on day 8 of the experiment, variations in the amounts of particulate organic carbon (POC) and particulate organic nitrogen (PON) and the C:N ratios of the settling particles in the bags also indicated rapid lithification processes (Fig. 7). The two sediment traps installed in each of the bags were only 1 m apart, but changes in the concentrations of POC and PON were already evident. The amounts of carbon and nitrogen in particles collected at the 2-m layer were lower than those in particles collected at the 1-m layer. This indicated that rapid lithification of settling particles could induce dynamic changes in the properties of particles.

Effects of biological activities on the removal of pollutants by particulates

Time variations in the transfer of Cu added to the control and eutrophication tanks in the MERL experiment are depicted in Fig. 8. The theoretical curve was computed according to the equation

$$[1] \quad C = (C_0 - k)e^{-\lambda t} + k$$

where C_0 is the initial concentration of Cu added, t is time, λ is the renewal rate of the seawater (4% per day), and k is the concentration of Cu in the renewed seawater from the bay.

The Cu added to the two tanks was nonconservative and each tank lost about half of the Cu during the first 24 h at about the same rate. Chemical analyses of particles obtained from sediment traps during the same time intervals indicated that particulate Cu in the control tank increased by six times, and that in the eutrophication tank by four times. In addition, 65–70% of the additional Cu was in the oxidizable phase. Estimates based on both the collection rate of the mainly settling and resuspended particles in the lower sediment trap in the control tank and the increment of Cu on the particles suggested that removal of Cu from the water column of the control tank could be attributed to scavenging by resuspended particles. Organic compounds released by organisms facilitated rapid binding of added Cu onto resuspended particles and accelerated scavenging of Cu substantially. However, the collection rate of particles in the eutrophication tank was only one-tenth of that in the control tank, so the effect of resuspension in the eutrophication tank was much reduced.

During the first 24 h, scavenging of Cu by resuspended particles accounted for only 20% of the total Cu removed from the water column of the eutrophication

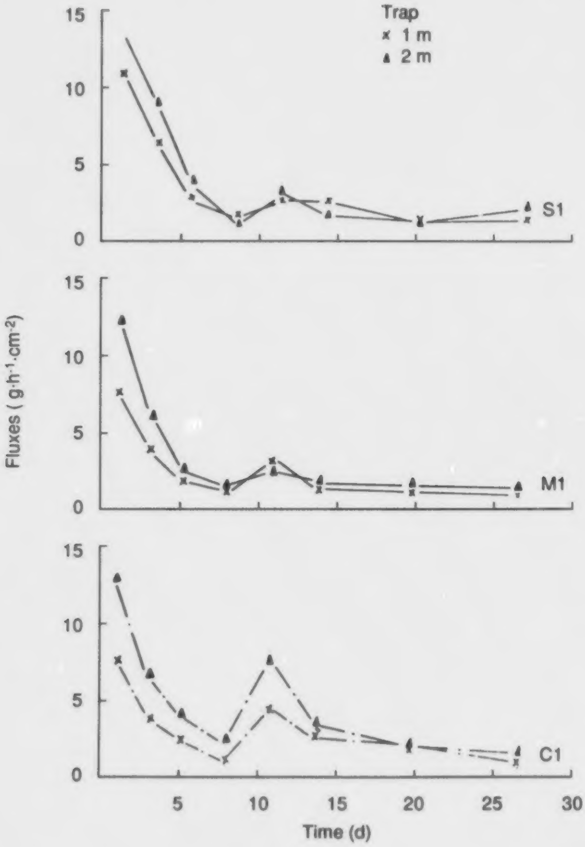


Fig. 9. Time variations of particulate fluxes in MEEE bags C1, M1, and S1.

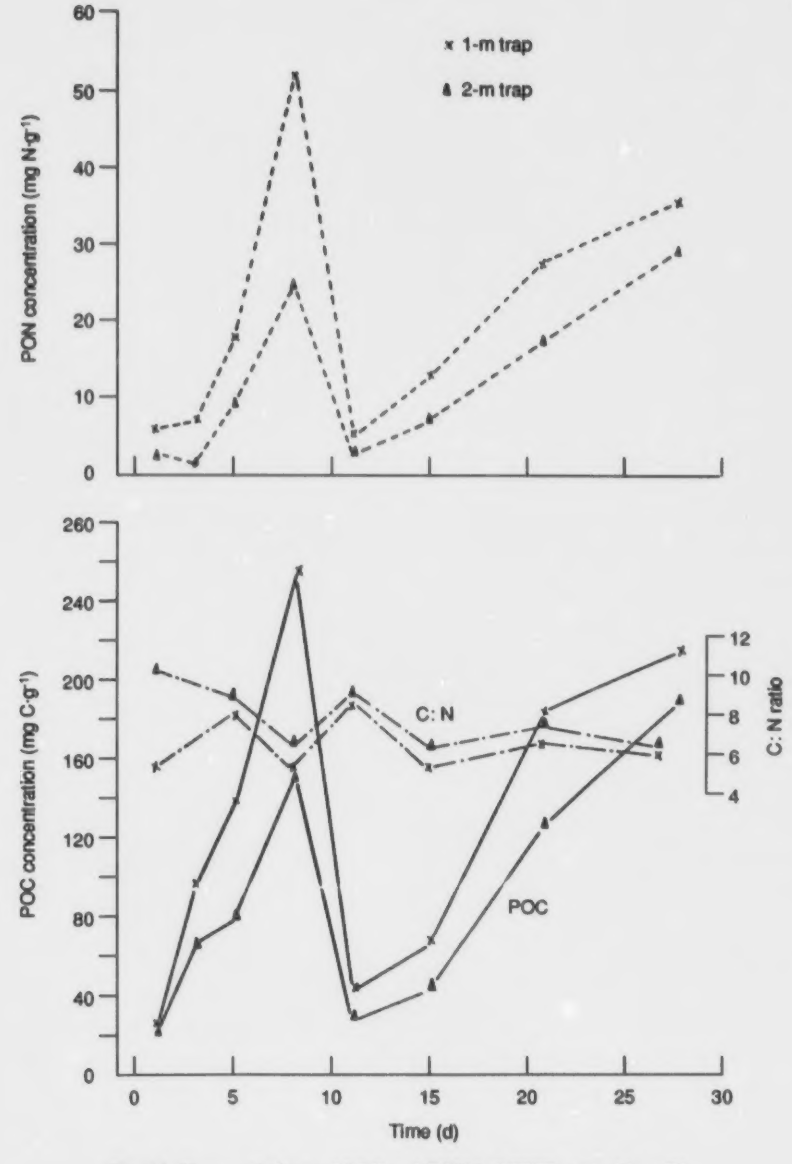


Fig. 10. Time variations of POC and PON in MEEE control bag C1.

tank. In this tank, removal of Cu was probably accomplished by filter-feeding of benthic organisms as indicated by the relatively larger benthos and more severe response to Cu toxicity later by these organisms. In summary, the MERL experiment showed that the removal of externally added Cu from the water column was fairly rapid, and that, within 2 d, the primary productivity of phytoplankton

Table 3. Plasma emission spectrometry of particles in C1, S1, and M1.

Element (ppm)	Bag C1	Bag S1	Bag M1	Background
Si	418.6	295.5	684.1	897.2
Al	56.88	43.32	99.95	147.6
Fe	127.6	102.8	32.27	42.8
Ti	6.67	4.35	6.53	6.56
Mn	0.74	0.54	0.61	0.70
V	0.12	0.10	0.11	0.12

would basically return to normal. The pollutant was removed by the actions of particulate matter and the final sink of the pollutant was the sediment. Organisms were involved in the removal process both directly and indirectly.

MEEE results also suggested that the effects of pollutants in the water column are ephemeral. Figure 9 shows the variations in particulate fluxes with time in bags C1, M1, and S1. During the first few days, the flux measured in sediment-treated bag S1 was larger than that in C1 and M1. After day 8, particulate fluxes in each of the bags were similar, and particles were mainly biological materials.

Initially, the water column contained relatively more inorganic suspended particles and concentrations of POC and PON (Fig. 10) were inversely related to their fluxes. As biogenic particles gradually increased, so did POC and PON. On day 8, primary productivity in the bag reached its maximum, and the rapid increase in concentrations of POC and PON also supported the suggestion that the particles were composed mainly of biotic materials. At the same time, the C:N ratio (5.7 by weight) was close to the Redfield ratio. Changes in the other bags followed the same trends. Scanning electron microscope observations of particles from day 1 and day 8 also revealed the totally different compositions of particles.

Analytical results of POC and PON in settling particles and concentrations of Fe and Al in sediments in each bag (Table 3) were used to compare the effects of the various pollutants in the bags. Concentrations of POC and PON of settling particles in each bag (Fig. 7) followed the order C1 > S1 > M1, whereas their C:N ratios followed the reverse order. Furthermore, the effect of sediment on the biota in bag S1 might be less than that in bag M1.

Background levels of particulate Al and Si were relatively high, indicating the terrigenous nature of detrital materials (Table 3). During the experiment, there were changes in the concentrations of various metals in particles relative to their respective background levels in all of the bags. These changes were the result of biological activities and lithification processes. In all cases, concentrations of particulate Al and Si decreased, whereas those of particulate Fe increased and those of particulate Mn, Ti, and V remained basically the same. The magnitude of decreases in Al and Si followed the order S1 > C1 > M1, whereas the magnitude of the increase in Fe followed the order C1 ≈ S1 > M1. Moreover, variations in metal content of particles collected from S1 at the end of the experiment were similar to those observed for particles collected from C1; the toxicity of heavy metals to organisms in S1 might be minimal.

Conclusions

Different nutrient levels in marine ecosystems not only affect the total biomass of the ecosystem but also induce changes in the community structure of the ecosystem. At the same time, the changes also alter the composition and properties of particulate materials.

Rapid lithification and oxidation of the organic fraction in settling particles also alter bonding properties between a given metal and the particles. Direct or indirect actions of organisms with respect to the transfer or removal of metallic pollutants should be investigated further.

Nixon, S.W., Pilson, M.E.Q., Oviatt, C.A., Donaghay, P., Sullivan, B., Seitzinger, S., Rudnick, D., Frithsen, J. 1984. Eutrophication of a coastal marine ecosystem — An experimental study using the MERL microcosms. *In* Fasham, M.J.R., ed., Flows of energy and materials in marine ecosystems. Plenum Publishing, New York, NY, USA. 105–135.

Biological Implications of Organic Carbon and Nitrogen in the Xiamen Enclosures

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Organic carbon and nitrogen in suspended and settled matter from Xiamen Marine Ecosystem Enclosed Experiments (MEEE) bags were determined in 1985 and 1986. Linear correlations between organic carbon and nitrogen were found in both suspended and settled matter. Regression lines had almost identical slopes and their intercepts passed near the origin. This suggested that the proportion of organic carbon and nitrogen in nonliving material was probably the same as that in living phytoplankton. Organic carbon and nitrogen were correlated with chlorophyll a in all enclosures. Organic carbon and nitrogen were both significantly correlated with primary productivity in enclosures treated with trace metals, but had poor correlations in enclosures treated with sediment. This implied that light extinction caused by sediment particles had a significant effect on primary production in the bag treated with sediment.

The Marine Ecosystem Enclosed Experiments (MEEE) in Xiamen was a cooperative program between Canada and China aimed at studying the transfer and fate of low concentrations of pollutants in a marine environment and their effects on the structure and function of a marine plankton community in Xiamen Bay.

Bags of woven polyethylene, coated with a plastic film on the inner surface, were used to enclose part of a natural column of seawater as experimental entities. Two concentrations of a trace-metal mixture and moderately polluted sediment, oil dispersant and chemically dispersed crude oil, and a control were studied. Detailed descriptions of the Xiamen MEEE facilities are given by Fu et al. (1989) and Wu J. et al. (this volume). During the 1985 and 1986 experiments, particulate organic carbon (POC) and particulate organic nitrogen (PON) in both suspended and settled materials were determined on a routine basis. The results of these determinations and their biological and biogeochemical implications are discussed in this paper.

Materials and methods

The experimental design and sampling methods are described in detail in Fu et al. (1989). In the 1985 experiment, bags C1 and C2 were the controls, receiving no additions of pollutants. Bags M1, M2, and M3 were treated with trace metals receiving concentrations of a mixture of Cd, Cu, Hg, Pb, and Zn. Concentrations of trace metals added to bags M1 and M2 were about 10 times the background levels normally measured in Xiamen Bay seawater, whereas bag M3 received concentrations of the trace metals at levels about 50 times the background level. Bags S1, S2, and S3 were treated with polluted sediment. The final sediment concentration in bags S1 and S2 was 11.2 ppm; in bag S3, the final sediment concentration was 112 ppm.

Materials retained by a glassfibre filter (Whatman GF/C, with a mean pore size of 1.2 μm) under low vacuum (25.3 kPa) were defined as "particulates." Concentrations of POC and PON were determined by standard methods (Grasshoff et al. 1983; Parsons et al. 1984). Briefly, 2 L of seawater was subsampled from the integrated seawater sample from 0–3 m and filtered under low vacuum through a preweighed glassfibre filter (Whatman GF/C, 47- or 55-mm) previously precombusted at 450°C for more than 4 h. The filter was then rinsed with a small volume of distilled water and dried in an oven at 50°C for 24 h. The weight difference of the dry filter before and after filtration gave the dry weight of the suspended matter. Organic carbon and nitrogen contents in suspended matter were then measured using a CHN analyzer at 800°C (Perkin-Elmer Elemental Analyzer Model 240C). An aliquot of sediment was thoroughly mixed, resuspended, and analyzed following the same method. Chlorophyll *a* and ^{14}C primary productivity data were taken from the MEEE Group's unpublished report (MEEE–85–Xiamen).

Results and discussion

The enclosed ecosystem provides a useful system for studying the biogeochemistry of organic carbon and nitrogen and their relationship with other biological parameters, such as chlorophyll *a* and primary productivity. An enclosed water column can reduce the impact of terrigenous materials transported into the sea by rivers or from the atmosphere. Thus, the production of phytoplankton becomes the major source of POC and PON in such an ecosystem. Because settled materials can be removed periodically, the effect of settled matter hydrodynamically resuspended and transformed and released in diagenesis can be minimized.

In the 1985 experiment, photochemical reactions were negligible and bacterioplankton responded in a similar manner in all bags (MEEE Group's unpublished report). Because it was impossible to ensure identical composition and structure of the zooplankton communities in each bag, results were compared only for those bags containing similar species of herbivorous zooplankton (i.e., bags C2, M2, M3, S1, and S3). POC and PON concentrations in the control bags reached their maximums between days 6 and 12, probably as a result of phytoplankton blooms (Figs 1 and 2). Inorganic nutrients (nitrate and phosphate) were also depleted during this period (Qian et al., this volume). Compared with the controls, fewer changes in POC and PON were observed in the bags treated with trace metals. In the two bags treated with low concentrations of metals, other than a 4-d delay in phytoplankton

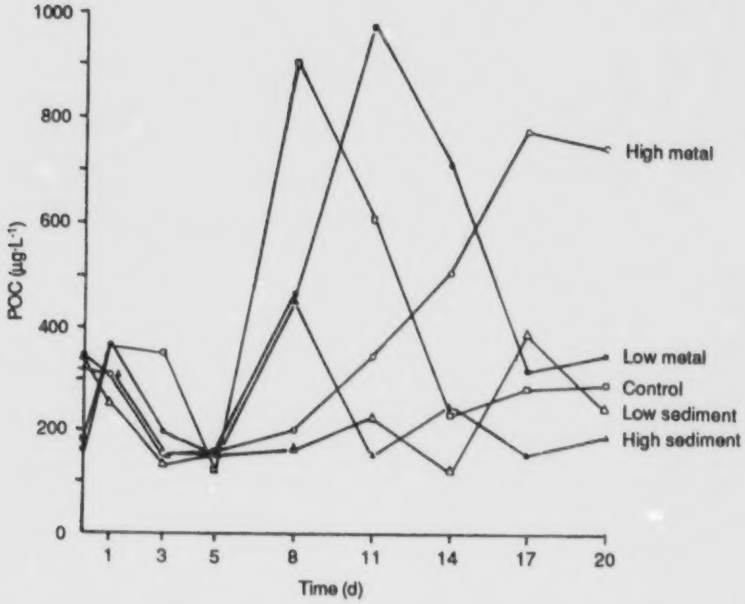

Fig. 1. Particulate organic carbon (POC) content in suspended material from 1985 experimental enclosures.

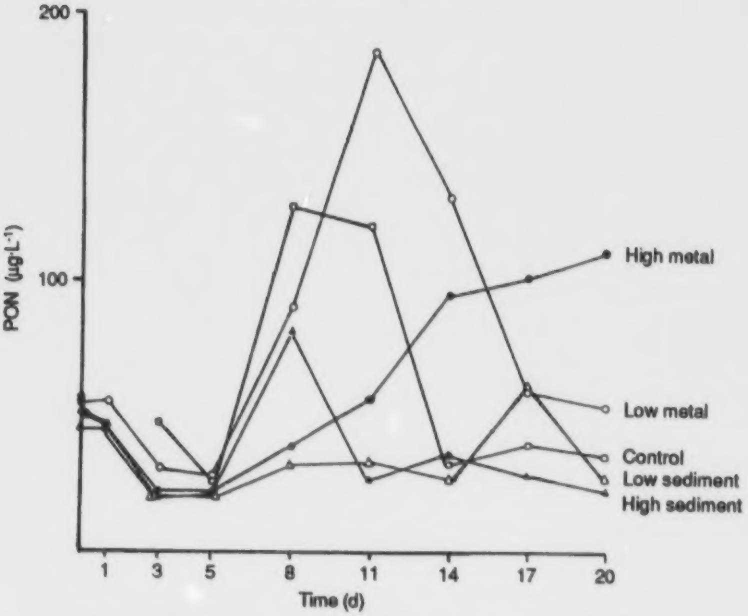

Fig. 2. Particulate organic nitrogen (PON) contents in suspended material from 1985 experimental enclosures.

blooms, no differences were detected; in the bag treated with high concentrations of metals, on the other hand, the phytoplankton bloom was delayed for about 6 d and the rate of increase of the bloom was slower than that in the control enclosures.

These results suggest that a mixture of heavy metals in low concentrations had almost no influence on primary production, but high concentrations of heavy metals could significantly delay biological activity and the formation of phytoplankton blooms (for more details on biological changes, see Qian et al. (this volume)). In contrast, primary production in bags treated with high and low concentrations of sediment was severely suppressed and no significant phytoplankton blooms were observed. Because there was no obvious release of toxic compounds from the sediment, the effects can reasonably be explained as having resulted from a reduction in light penetration caused by sediment particles, which reduced the growth of the phytoplankton. A similar conclusion can be deduced based on the similar patterns observed in the bags for chlorophyll *a* and primary productivity (Fu et al. 1989).

Chlorophyll *a* is an index of phytoplankton biomass and generally it correlates well with POC and PON (Fogg 1975). In fact, positive correlations between POC and chlorophyll *a* have been found in field investigations (Parsons 1975; Verlencar and Qasim 1985), and linear regression equations have been used to estimate the relative proportion of phytoplankton and detrital organic matter (Verlencar and Qasim 1985). Primary productivity determined using the ^{14}C method shows a more complicated relationship with POC and PON. The coefficients of linear regression equations among ^{14}C primary productivity, chlorophyll *a*, POC, and PON for the 1985 experiment are summarized in Table 1. Positive correlations among POC, PON, and chlorophyll *a* were obtained for all experimental bags with the exception of bag S1.

With respect to the relationship between POC, PON, and ^{14}C primary productivity, clear differences existed between those bags treated with sediment and the other bags. In the control and the bags treated with metals, significant positive correlations were observed; whereas in the bags treated with sediment, no definitive correlations were found. The results implied that the main factor regulating primary production in bags treated with different pollutants was not the same. It appears that the decrease in light intensity in bags treated with sediment significantly suppressed the photosynthetic rate.

The C:N ratio is a useful geochemical parameter that can be calculated from organic carbon and nitrogen data. The C:N ratio in marine sediments generally increases with depth (Romankevich 1984), suggesting higher degradation and mineralization rates of organic nitrogen in diagenesis than those of organic carbon. However, the records in the sediment do not reflect the initial changes of dead organisms. The enclosures, with no interference from terrigenous organic matter (C:N ratio generally over 10:1) and resuspended sediment, can also provide some information on initial diagenetic changes. In the 1985 study, POC:PON ratios in suspended material and sediment were very similar, 6.4:1 and 6.3:1, respectively, and regression lines had intercepts at or near the origin (Figs 3 and 4). Suspended material was composed of living plankton and the remains of dead organisms, whereas settled material was mainly composed of detrital matter. The similar C:N ratios implied a similar C:N ratio in living and nonliving organic matter, and that immediately after the death of organisms, the C:N ratio remained unchanged during

Table 1. Regression formula and coefficients among particulate organic carbon (POC), particulate organic nitrogen (PON), and chlorophyll *a* and primary productivity.

	Regressions of POC and PON with chlorophyll *a*						Regressions of POC and PON with primary productivity					
	POC = *b* + *a*		Ch[a]	PON = *b* + *a*		Ch[a]	POC = *b* + *a*		Pr[b]	PON = *b* + *a*		Pr[b]
Bag	*b*	*a*	(*r*)	*b*	*a*	(*r*)	*b*	*a*	(*r*)	*b*	*a*	(*r*)
C1	119	29	0.815	25.4	3.36	0.878	146	17.3	0.701	30.5	1.76	0.670
C2	239	20	0.592	31.9	4.30	0.801	206	12.7	0.835	27.5	2.31	0.942
M1	90	45	0.937	9.0	9.68	0.964	191	15.5	0.797	29.1	3.49	0.864
M2	222	29	0.916	33.9	5.99	0.932	199	38.6	0.735	28.3	8.07	0.763
M3	101	40	0.868	21.6	5.72	0.898	172	32.6	0.906	34.5	4.26	0.850
S1	339	-48	-0.387	46.4	-5.15	-0.254	222	-0.57	-0.016	36.0	-0.68	-0.116
S2	-45	82	0.878	-3.98	12	0.839	131	11.1	0.239	23.9	0.99	0.140
S3	96	49	0.888	10.5	9.41	0.929	197	11.5	0.391	27.4	2.86	0.529

[a] Ch = chlorophyll *a*.

[b] Pr = ^{14}C primary productivity.

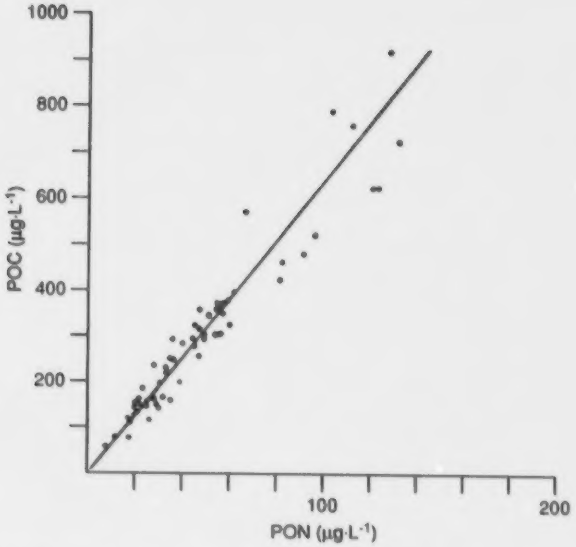

Fig. 3. Linear regression of particulate organic carbon (POC) and particulate organic nitrogen (PON) in suspended material for the 1985 experiment.

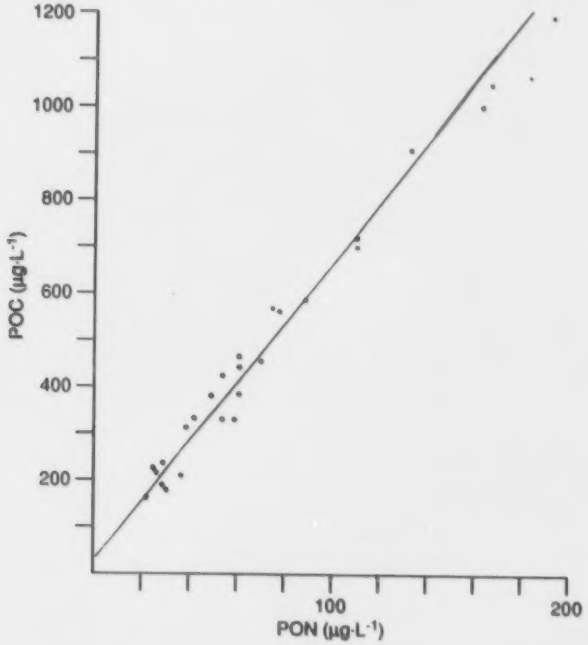

Fig. 4. Linear regression of particulate organic carbon (POC) and particulate organic nitrogen (PON) in settled material for the 1985 experiment.

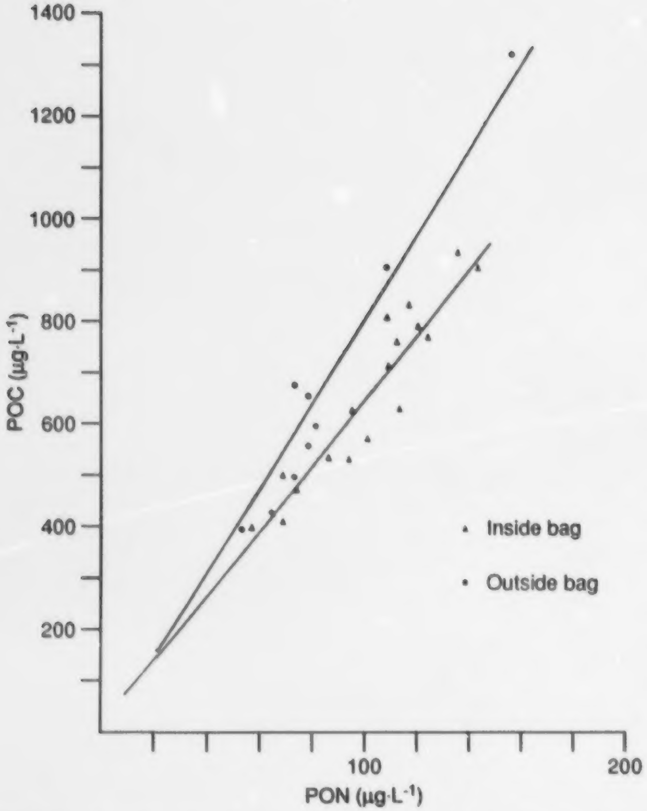

Fig. 5. Linear regression of particulate organic carbon (POC) and particulate organic nitrogen (PON) in suspended material for the 1986 experiment.

the initial stage of biodegradation. A similar result was reported by Menzel and Ryther (1964) for surface seawater.

Figure 5 shows the C:N ratios obtained in the 1986 experiment. Both the control bag and the bags treated with Corexit 9527 had comparable C:N ratios of 6.1:1. The C:N ratio inside the bags was lower than that outside the bags (natural seawater column, C:N = 7.9:1), but the ratios were close to those determined from the 1985 results. The site for the 1986 experiment had strong tidal mixing and, consequently, resuspension of bottom sediment. It is suspected that resuspension of sediment with a high C:N ratios resulted in higher C:N ratio for particulate materials in the water column.

Examination of the results from the two experiments suggested that identical changes occurred in the C:N ratio during initial degradation of dead organisms. After depositing on the seabed, further biological and chemical degradation would lead to the fractionation of organic carbon and nitrogen and result in a higher C:N ratio in the sediment than in the biota.

Acknowledgments

We express our sincere thanks to the International Development Research Centre of Canada and the State Oceanic Administration of China for their financial support, and to the Laboratory Centre of Xiamen University for POC and PON determinations.

Fogg, G.E. 1975. Primary productivity. *In* Riley, J.P., Skirrow, G., ed., Chemical oceanography — volume 2 (2nd ed.). Academic Press, London, UK. 384–444.

Fu, T., et al. 1989. Xiamen marine enclosed ecosystem experiment and its application in marine environment protection studies. Environmental Sciences in China, in press. [In Chinese.]

Grasshoff, K., Ehrhardt, M., Kremling, K. 1983. Methods of sea water analysis. Verlag Chemie, Germany.

Menzel, D.W., Ryther, J.H. 1964. The composition of particulate organic matter in the western North Atlantic. Limnology and Oceanography, 9, 179–186.

Parsons, T.R. 1975. Particulate organic carbon in the sea. *In* Riley, J.P., Skirrow, G., ed., Chemical oceanography — volume 2 (2nd ed.). Academic Press, London, UK. 365–381.

Parsons, T.R., Maita, Y., Lalli, C.M. 1984. A manual for biological and chemical methods for seawater analysis. Pergamon Press, Oxford, UK.

Romankevich, E.A. 1984. Geochemistry of organic matter in the ocean. Springer-Verlag, Berlin, Germany.

Verlencar, X.N., Qasim, S.Z. 1985. Particulate organic matter in the coastal and estuarine waters of Goa and its relationship with phytoplankton. Estuarine and Coastal Shelf Science, 21, 235–243.

Ecological Impacts of Pollutants on Particulate Organic Carbon, Nitrogen, and Phosphorus in Marine Ecosystem Enclosed Experiments

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Particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP) were determined in the marine ecosystem enclosed experiments (MEEE) launched during the summer of 1985 at Saanich Inlet, BC, Canada. Time variations of POC, PON, and PP in the control were fairly consistent. The ratios of POC:PON and POC:PP were relatively stable at 6.0:1 ± 0.61 and 95:1 ± 19.9 respectively. The ratios were in good agreement with those measured in natural seawater in Saanich Inlet and in the open sea. Ratios of POC to chlorophyll a *were less than 100:1. In the dark enclosure to which sediment had been added, phytoplankton declined greatly, corresponding with reductions in POC, PON, and PP. A very high ratio of POC to chlorophyll* a *suggested that the particulate matter was composed mainly of detritus. The higher value of POC:PON (average of 8:1) and the lower value of POC:PP (average of 58:1) displayed the following sequence of biological degradation: N > C > P. In the enclosure to which sulfide had been added, POC, PON, and PP increased significantly from day 5 to the end of the experiment. Lower ratios of both POC:PON (<5:1) and POC:PP (<80:1) displayed the characteristics of bacterial growth. In enclosures to which sediment only and sediment plus silt had been added, variations of POC, PON, and PP were consistent with those of chlorophyll* a *and* ^{14}*C productivity. During diatom blooms, ratios of both POC:PON and POC:PP increased with simultaneous reductions in nitrate-N and phosphate-P in the water column.*

Over the last decade, many types of marine ecosystem enclosed experiments (MEEEs) have been used to investigate the ecological effects, biogeochemical behaviour, and fate of marine pollutants (Grice and Reeve 1982). Similarly, coastal and harbour dredging have received considerable attention because contaminated sediments are often resuspended as a result of dredging operations, thereby releasing adsorbed pollutants. An experiment designed to study the release of pollutants from sediments and their effects on ecosystems under different conditions was carried out at Saanich Inlet, BC, Canada, during the summer of 1985 using a catamaran supporting several enclosures. This research project was jointly sponsored by the International Development Research Centre of Canada and the State Oceanic Administration of the People's Republic of China.

This paper discusses the effects of contaminated sediments on the composition of particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP).

Materials and methods

The experimental enclosures are supported on a catamaran barge (Fig. 1). It is constructed with aluminum supports and two parallel floats and is capable of holding five fibreglass tanks (each tank is 1 m in diameter and 2 m deep). All five tanks were used, each receiving a different treatment. Tank 1 was the control, containing seawater pumped from 17 m below the surface using a peristaltic pump. About 100 L of False Creek (near Vancouver, BC) sediment moderately polluted by heavy metals was added to each of the other tanks. Seawater was pumped into these tanks as it was in tank 1. To stop phytoplankton growth, the tanks were covered with black polyethylene sheets until the first sampling session 1 d later.

The following coastal environments were simulated in this study (see Whitney 1985 for details): tank 1 — no sediment, full sunlight (control); tank 2 — sediment bed, full sunlight; tank 3 — sediment bed, no sunlight (black cover); tank 4 — sediment bed, high silt load (to show its scavenging capacity); and tank 5 — sediment bed, anoxic water (Na_2S added).

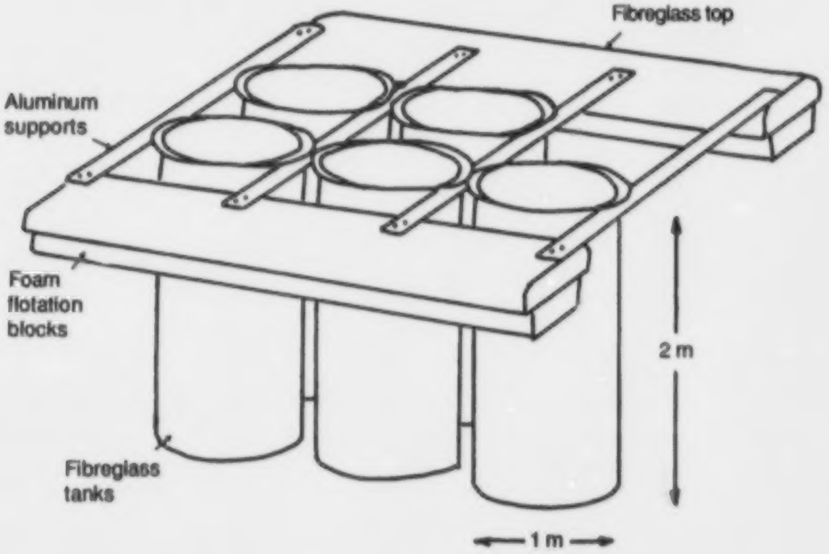

Fig. 1. Arrangement of experimental enclosures.

During the experiment, all of the tanks were covered with clear or black polyethylene sheets to restrict contamination by airborne particles. Sampling was carried out between 13 August and 6 September 1985.

About 2 L of seawater was filtered through a 47-mm precombusted glass fibre filter (Whatman GF/C). The filters were dried in an oven at 60°C for 24 h. Concentrations of POC and PON were measured using a Perkin-Elmer Model 240 elemental analyzer (Parsons et al. 1984). For particulate phosphorus determinations, 500 mL of seawater was filtered through a 24-mm precombusted glass fibre filter (Whatman GF/C) and the content was determined by colorimetry (Solorzano and Sharp 1980).

Results and discussion

In the control (tank 1), ranges of POC, PON, and PP were close to natural values measured in the seawater of Saanich Inlet at the same time (Table 1) and showed the characteristic high productivity associated with coastal seawater (Sharp 1983). Variations in POC, PON, and PP were similar, with peaks on 15, 21, and 30 August (Fig. 2d). Ratios of C:N and C:P were stable (Fig. 2b). Average C:N and C:P ratios in tank 1 were close to the field ratios measured in Saanich Inlet seawater (Table 1) and were slightly less than the Redfield ratios for phytoplankton (C:N:P = 106:16:1) (Redfield 1934). The relative stability of the ratios in tank 1 suggested that the composition of phytoplankton remains relatively stable even when productivity is low. In the control, particulates were derived mainly from phytoplankton and weight ratios were representative of a relatively unpolluted area, such as Saanich Inlet.

In tank 2, heavy metals (Cd, Cu, Pb, and Zn) in contaminated sediment were released (Fig. 3). The time variation of chlorophyll *a* showed that the phytoplankton bloom was delayed for about 1 week. At the same time, the biomass increased significantly as nutrients were released from the sediment. These results are similar to those reported from the 1984 Marine Ecosystem Enclosed Experiments (MEEE) (Parsons et al. 1986) in which mine tailings were added to the enclosures. The peaks of PON and POC on 21 and 23 August, respectively, coincided with the diatom bloom. It was followed by two flagellate blooms: the first between 23 and 26 August, overlapping the diatom bloom, and the second beginning on 3 September, coinciding with the second peaks of POC and PON. Ratios of C:N between of 17 August and 6 September were about 6:1 (Fig. 3), indicating that the particulate in tank 2 has the characteristics of phytoplankton. The higher C:N ratio (9.4:1) on

Table 1. Ranges of particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP) ($\mu mol \cdot L^{-1}$) and ratios of C:N and C:P in the control (tank 1) and in the seawater of Saanich Inlet, British Columbia.

	Control	Saanich Inlet
POC	8.1–44.8	13.9–65.6
PON	1.2–6.8	2.1–8.3
PP	0.11–0.46	0.07–0.51
C:N	6.0	5.9–8.0
C:P	95.4	109–199

23 August corresponded with the lowest levels of nitrate and ammonium, suggesting that the diatom bloom was approaching nitrogen limitation (C:N = 10:1) (Goldman and McCarthy 1978) with depleted nutrients.

In tank 2, the PP peak occurred before the phytoplankton bloom; therefore, the C:P ratio increased (203:1–282:1) when the bloom occurred. This observation suggested an increase in the rate of uptake of phosphorus by phytoplankton, resulting

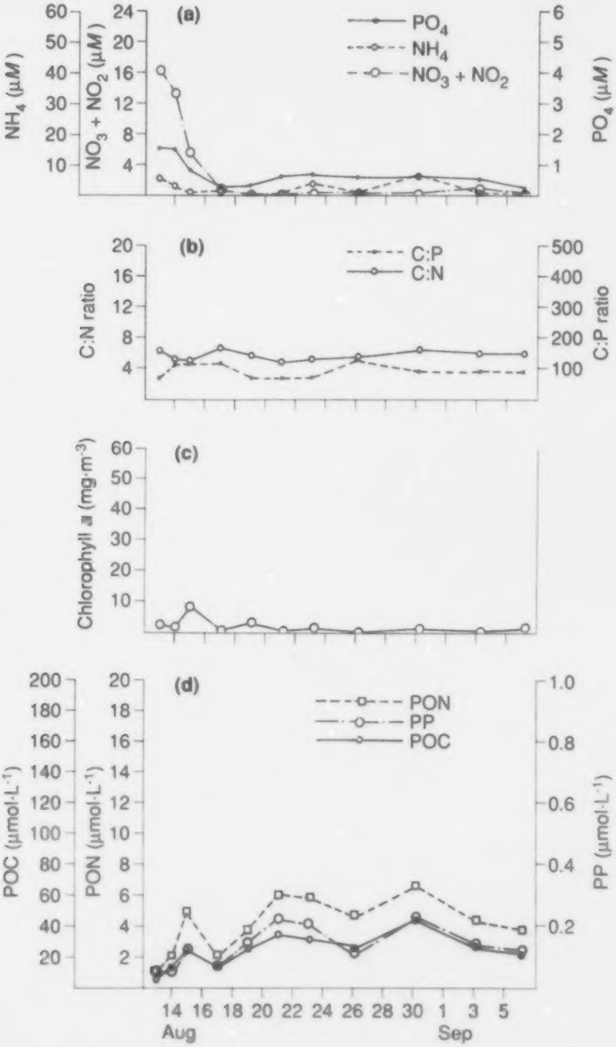

Fig. 2. Time variation of (a) nutrients, (b) C:N and C:P ratios, (c) chlorophyll *a*, and (d) particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP) in tank 1.

in the decrease in phosphorus in phytoplankton cells and the increase in the C:P ratio during the bloom. At the beginning of the experiment, there were higher C:N ratios and higher PP in tanks 3, 4, and 5. The similar trends suggest that particulates in these tanks probably originated from the polluted sediment used in the experiments.

In the dark enclosure (tank 3), although levels of nutrients were high,

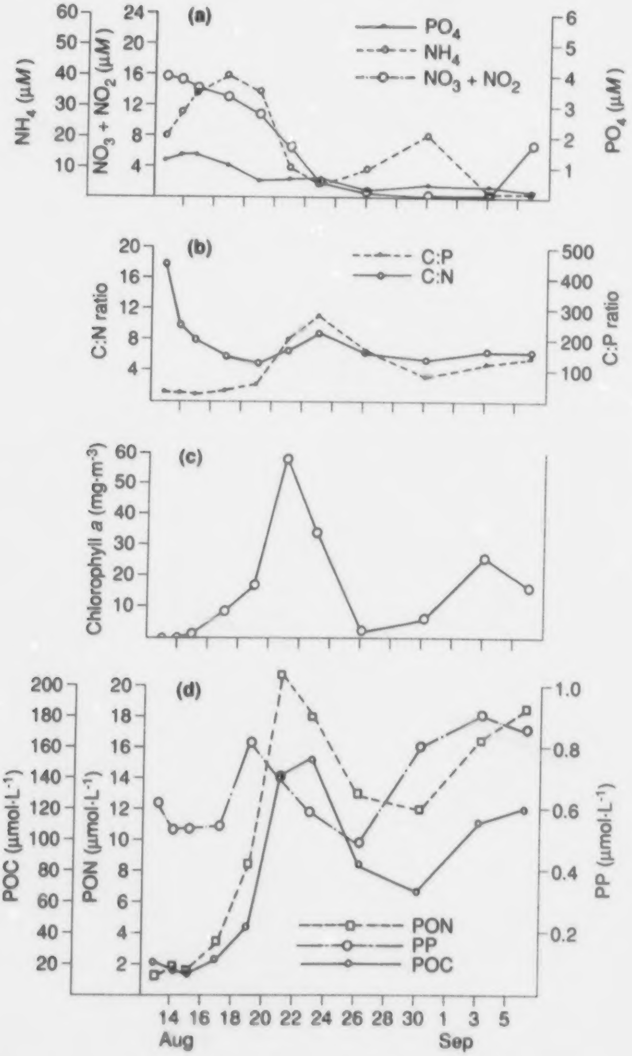

Fig. 3. Time variation of (a) nutrients, (b) C:N and C:P ratios, (c) chlorophyll *a*, and (d) particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP) in tank 2.

phytoplankton growth was suppressed significantly. Throughout the entire experiment, no peaks of chlorophyll *a* were observed (Fig. 4c), probably because of the low sensitivity of the method used. Peaks of POC and PON were similar to those in the control on 17 August and corresponded with the small bloom of diatoms (about 1 219 cells·mL^{-1}). On 3 September, POC, PON, and PP increased slightly, corresponding with the small flagellate bloom. Thus, any one of these three parameters can be used as an indicator of phytoplankton growth. During the period of

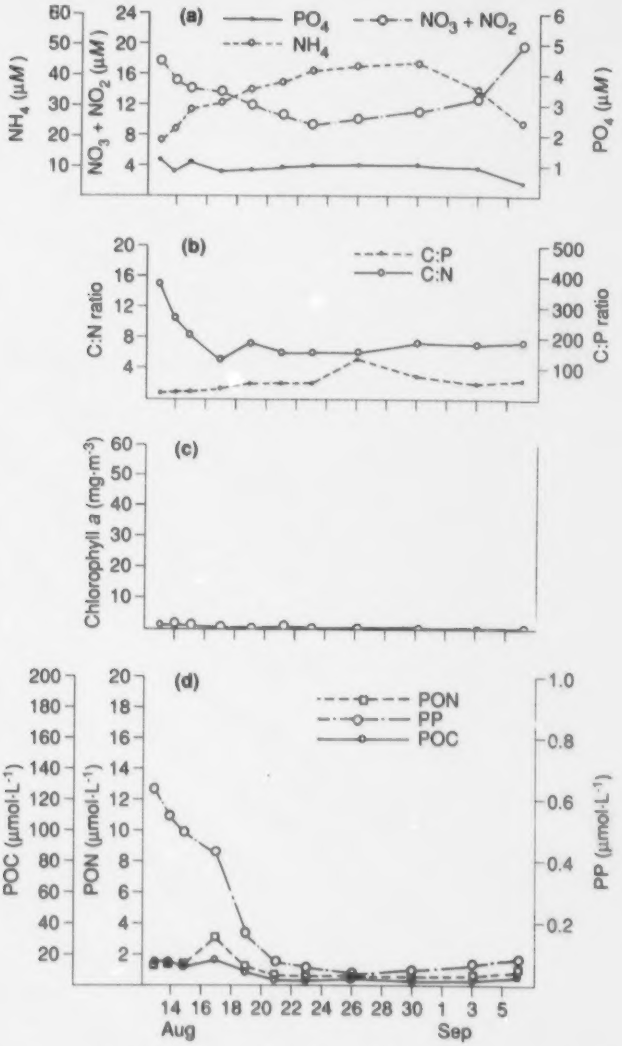

Fig. 4. Time variation of (a) nutrients, (b) C:N and C:P ratios, (c) chlorophyll *a*, and (d) particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP) in tank 3.

phytoplankton growth, C:N ratios were about 7:1, higher than those in the control (C:N = 6:1). However, C:P ratios were about 65:1, lower than those in the control (C:P = 95:1). The low C:P ratios might have been a result of the following:

- The short-duration diatom bloom under suboptimal conditions was rapidly succeeded by flagellates, which possessed higher C:N and lower C:P ratios; and

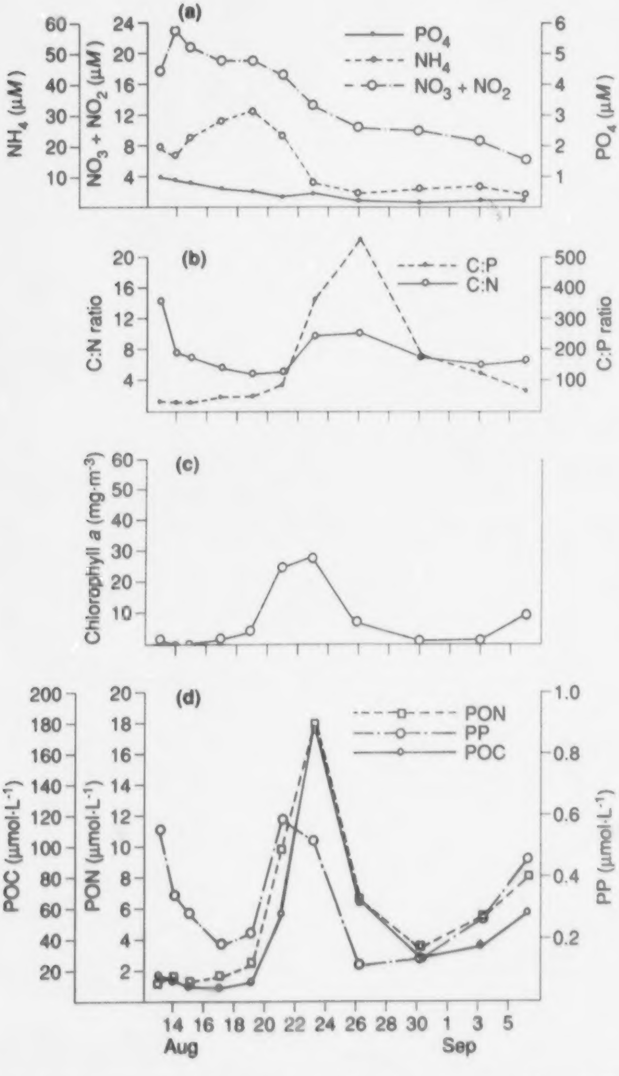

Fig. 5. Time variation of (a) nutrients, (b) C:N and C:P ratios, (c) chlorophyll *a*, and (d) particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP) in tank 4.

- The particulates were mainly composed of phytoplankton detritus (Parsons 1975) as primary productivity was nearly zero and the POC:chlorophyll *a* ratio was very high (about 3 850:1).

In tank 4, the enclosure treated with sediment and silt, levels of heavy metals in the water changed only slightly, but nitrate-N concentrations increased significantly (Fig. 5). Variations of POC, PON, PP, and chlorophyll *a* with time were similar to those occurring in tank 2, but their peaks were delayed even further. This delay was probably caused by the silt particles reducing light penetration. Individual peaks of PP (21 August) and POC and PON (23 August) reflected the diatom bloom. After the lowest point, on 30 August, a second peak appeared along with the flagellate bloom. The diatom bloom in this tank showed higher and sharper peaks than those in tank 2. The sharp peak was consistent with the very short growth period for diatoms (Fig. 6). The C:N ratios on 23 and 26 August were greater than 10:1 (Fig. 5), suggesting a nitrogen-limiting condition. On the other hand, dominant species of

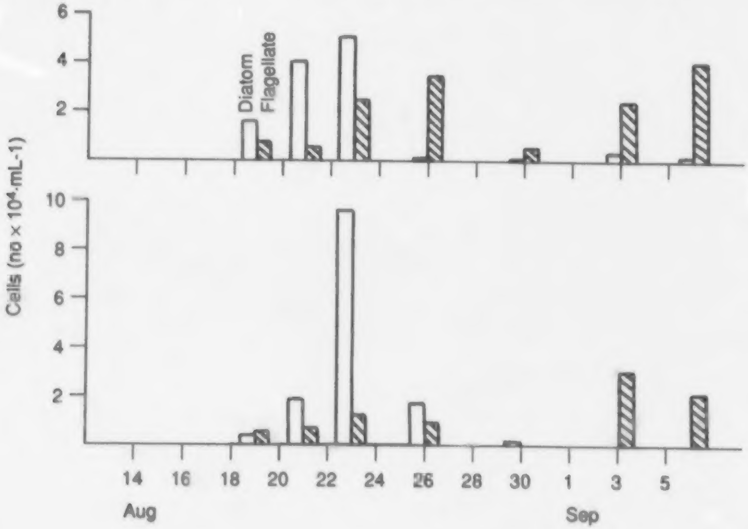

Fig. 6. Time variation of phytoplankton biomass.

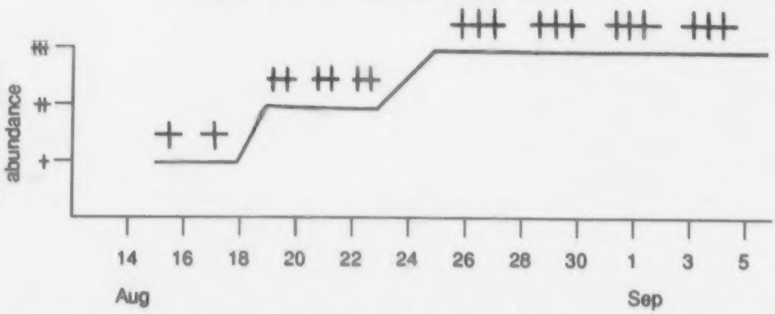

Fig. 7. Time variation of bacteria in tank 5.

diatoms in tanks 2 and 4 differed. *Thalassiosira* spp were dominant in tank 2, whereas *Skeletonema costatum* was dominant in tank 4. It appears that the dominant species were related to the nutrient composition of the seawater. *Thalassiosira* spp are bigger and contain more nitrate in their cells (Conover 1975), whereas *Skeletonema costatum* contains less nitrate per cell (Dortch 1982). The time variation of PP in tank 4 was also similar to that in tank 2, i.e., the PP peak occurred

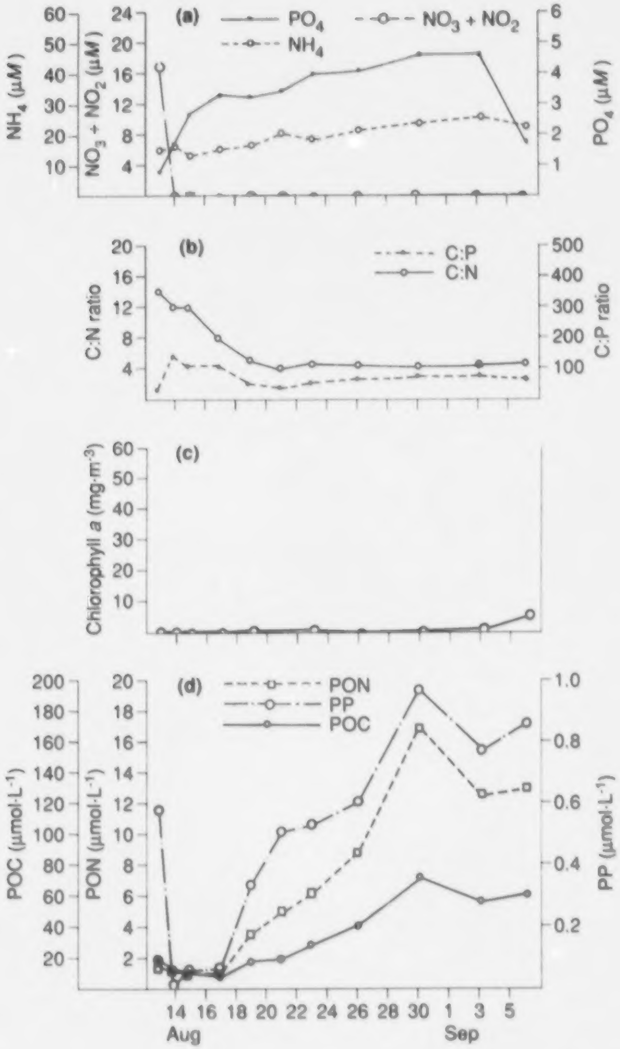

Fig. 8. Time variation of (a) nutrients, (b) C:N and C:P ratios, (c) chlorophyll *a*, and (d) particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP) in tank 5.

before the phytoplankton peak, indicating a rapid uptake of phosphorus and a high C:P ratio (573:1).

In the enclosure to which sulfide was added (tank 5), both nitrate- and nitrite-N were reduced. Diatoms and flagellates were not observed until 3 September. However, bacteria were present on 15 August and increased continuously with time. POC, PON, and PP also increased with time, corresponding with the increase in bacteria (Figs 7 and 8). The C:N and C:P ratios decreased constantly with time until they reached values of C:N = 5:1 and C:P = 80:1 on 17 August. Otsuki and Hanya (1968) have stated that the C:N ratio in bacteria could be less than 5:1. Meanwhile, Harrison et al. (1977) report that, in coastal waters, the uptake of phosphorus by bacteria could be very important. The abundance of bacteria is a plausible explanation for the low C:N and C:P ratios in the particulates. After 3 September, the diatom and flagellate blooms corresponded with increases in C:N and C:P ratios, declines in nutrients, and increases in POC, PON, PP, and chlorophyll *a*.

Acknowledgments

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Conover, S.A.M. 1975. Partitioning of nitrogen and carbon in cultures of the marine diatom *Thalassiosira fluviatilis* supplied with nitrate, ammonium, or urea. Marine Biology, 32, 231–246.

Dortch, F.Q. 1982. Effect of growth conditions on accumulation in three marine diatoms. University of Washington, Seattle, WA, USA. PhD thesis.

Goldman, J.C., McCarthy, J.J. 1978. Steady-state growth and ammonia uptake of a fast-growing marine diatom. Limnology and Oceanography, 23, 695–730.

Grice, G.D., Reeve, M.R. 1982. Introduction and description of experimental ecosystems. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 1–10.

Harrison, W.G., Azam, F., Renger, E.H., Eppley, C.W. 1977. Some experiments on phosphate assimilation by coastal marine plankton. Marine Biology, 40, 9–18.

Otsuki, A., Hanya, T. 1968. On the production of dissolved nitrogen-rich organic matter. Limnology and Oceanography, 13, 1 983.

Parsons, T.R. 1975. Particulate organic carbon in the sea. *In* Riley, J.P., Skirrow, G., ed., Chemical oceanography. Academic Press, London, UK. 365–383.

Parsons, T.R., Maits, Y., Lalli, C.M. 1984. A manual of chemical and biological methods for seawater. Pergamon Press, New York, NY, USA.

Parsons, T.R., Thompson, P., Wu, Y., Lalli, C.M., Hous, S., Xu, H. 1986. The effect of mine tailings on the production of plankton. Acta Oceanologica Sinica, 5, 417–423.

Redfield, A.C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. *In* Daniel, R.J., ed., James Johnstone memorial volume. Liverpool University Press, Liverpool, UK.

Sharp, J.H. 1983. The distribution of inorganic nitrogen and dissolved and particulate organic nitrogen in the sea. *In* Carpenter, E.J., Capone, D.G., ed., Nitrogen in the marine environment. Academic Press, New York, NY, USA. 1–36.

Solorzano, L., Sharp, J.H. 1980. Determination of total dissolved and particulate phosphorus in natural waters. Limnology and Oceanography, 25, 754–758.

Whitney, F.W., ed. 1985. Metal fluxes from False Creek sediments under various chemical and biological conditions — Data Report MEEE 85-2. Institute of Ocean Sciences, Sidney, BC, Canada. 38 p.

Distribution of Heavy Metals in Xiamen Seawater and in the MEEE Enclosures

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Distributions of dissolved, and weakly and strongly bound particulate heavy metals in surface seawater from Xiamen Bay and Jiulongjiang Estuary were determined in a clean laboratory. It was found that most previous measurements, particularly of Pb and Zn, from the same areas have been overestimated.

In Xiamen Bay seawater, Co, Fe, Ni, Pb, and Zn existed mainly in the particulate phase, whereas Cd and Cu existed mainly in the dissolved phase. The partition coefficient (K_d) of each metal between the particulate and dissolved phases followed the order Fe > Co > Pb > Zn > Ni > Cu > Cd. In surface seawater from Jiulongjiang Estuary, dissolved Co, Cu, Pb, and Zn behaved conservatively, whereas Cd, Fe, and Ni did not. More than 90% of the riverborne suspended matter and its associated heavy metals settled in the estuary.

Advantages and limitations of the Marine Ecosystem Enclosure Experiment (MEEE) in studying the behaviour of heavy metals are discussed based on a comparison of field data with those obtained from the enclosure experiment.

Since the mid-1970s, improvements on analyses of trace metals in seawater have revealed probable errors in previous measurements (Boyle and Edmond 1975; Wong et al. 1977; Kanamori 1981). Table 1 lists changes in the concentrations of trace metals measured in seawater over the last 40 years. Improvements in analytical techniques are the result of employing contaminant-free methods that provide a more realistic picture of background levels and a better distribution of various trace metals in the ocean (Moore 1978; Bruland 1980; Boyle et al. 1981; Bruland and Franks 1983; Flegal and Patterson 1983; Magnusson and Westerland 1983).

In 1985, a clean laboratory was built in the Third Institute of Oceanography incorporating technology transferred from the Institute of Ocean Sciences in Canada. The laboratory makes it possible to monitor the concentration and distribution of trace metals in regional seawaters. Xiamen Bay connects with Jiulongjiang Estuary and faces the Taiwan Strait to the east. The annual flow of Jiulongjiang River exceeds 11.7×10^9 cm^3 and carries more than 2.5×10^6 t of silt and clay to the estuarine area.

This paper presents the results of studies on the concentration and distribution of

Table 1. Changes in the concentration (μg/kg) of trace metals in open ocean water over the last 40 years.

Reference	Cu	Pb	Zn	Cd	Co	Ni	Fe
Sverdrup (1942)	10	4	5	—	—	—	—
Goldberg (1965)	3	0.03	10	0.11	0.1	2	10
Riley (1975)	0.5	0.03	4.9	0.1	0.05	1.7	2
Kanamori (1981)	0.02–0.3	0.02–0.06	0.01–0.6	0.01–0.1	0.005	0.2–0.6	0.2–0.5
Bruland (1980)							
Surface	0.034	0.001	0.006	0.0003	0.007	0.146	0.008
Deep	0.130	0.0045	0.390	0.117	0.002	0.566	0.045

Table 2. Trace metal analysis of seawater (standard sample NASS-1) and blanks ($\mu g \cdot kg^{-1}$).

Values	Cu	Pb	Zn	Cd	Co	Ni	Fe
Reference	0.099±0.010	0.039±0.006	0.159±0.028	0.029±0.004	0.004±0.001	0.257±0.027	0.192±0.036
Measured	0.102	0.032	0.156	0.029	0.005	0.280	0.225
Analytical blank[a]	0.0060±0.0022	0.001±0.001	0.040±0.023	0.0014±0.003	0.001±0.001	0.015±0.005	0.007±0.020

[a] $n = 10$.

dissolved and particulate Cd, Co, Cu, Fe, Ni, Pb, and Zn in surface seawater of Xiamen Bay and Jiulongjiang Estuary. In addition, field data are compared with the behaviour of trace metals in enclosed seawater from the Xiamen Marine Ecosystem Enclosure Experiment (MEEE).

Methods

The 1985 MEEE data used in this study came from two 10 m^3 control bags (Li et al. this volume). Samples for the in-situ data (Fig. 1) were collected in August of the same year. Stations 1 and 2 were situated northeast of Xiamen Island (East Bay) where the input of industrial wastes is relatively low. Stations 3–8 were located on the western side of Xiamen Island (West Bay) where the annual input of industrial and domestic wastes is about 30×10^6 t. The other stations were situated within Jiulongjiang Estuary. Stations 9–11 were at the freshwater end, with salinities of less than 0.5‰; stations 24–26 were near the open sea (Outer Bay), with salinities greater than 30‰; and stations 12–23 were within the brackish area. Details of the sampling procedure are given in Li et al. (1987).

Each seawater sample was filtered through a 0.4-µm Nuclepore membrane filter under low vacuum. The filtrate was complexed with ammonium 1-pyrrolidine dithiocarbamate/diethyl ammonium diethyl dithiocarbamate (APDC/DDDC), extracted with Freon-TF, back-extracted with dilute nitric acid, and then analyzed using a graphite furnace flameless atomic absorption spectrophotometry (GFAAS) for dissolved metals (Li et al. 1987). Particulate matter retained on the filter was extracted with 0.5 *N* HCl for 24 h and the dissolved portion was analyzed using GFAAS for weakly bound metals. The remaining undissolved portion was further digested with aqua regia and HF to determine the strongly bound metals.

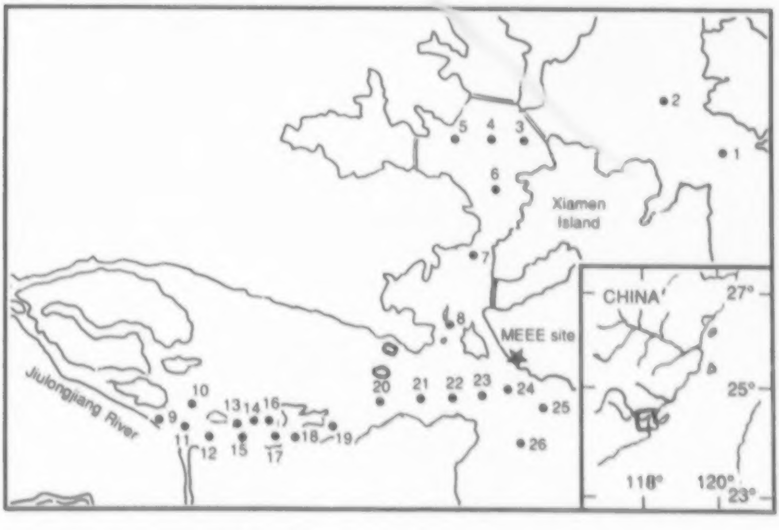

Fig. 1. Location of sampling stations (inset shows position of study area).

The results of the trace-metal analyses of the seawater standard and blank samples indicate that the analyses were reliable and that the blanks had very low levels of trace metals (Table 2).

Results and discussion

Concentration and distribution of dissolved metals

Characteristics of surface water from the Xiamen Bay area and concentrations of seven dissolved metals in the waters are presented in Table 3. All metal concentrations were fairly low. Regional variations were consistent with levels of pollution: the highest occurring in the West Bay and the lowest in the East Bay. The area including stations 3–8 was polluted by heavy metals, but at concentrations still within the ranges for coastal seawater. All values differed substantially from those reported earlier (Xu et al. 1986): i.e., before the clean laboratory procedure was used, Xu et al. (1986) could not detect any variation among the different regions. Compared with this study, their reported values for Pb and Zn were higher by two orders of magnitude, and Cu was four times higher; only the values for Cd were similar.

Changes in salinity, pH, and Eh were observed south of Xiamen Island at the mouth of Jiulongjiang River as fresh water mixes with seawater. There were corresponding variations in the speciation and concentration of heavy metals. Changes in the concentration of dissolved metals with salinity are shown in Fig. 2.

Dissolved Cu and Zn in the estuary behaved conservatively and their relations with salinity were

[1] $[\mathrm{Cu}]_d = -0.015[S] + 0.79, (r = 0.95; n = 19)$

[2] $[\mathrm{Zn}]_d = -0.012[S] + 0.45, (r = 0.90; n = 18)$

The concentrations of Co and Pb did not change significantly within the estuarine area, their values being 0.016 $\mu g \cdot kg^{-1}$ and 0.008 $\mu g \cdot kg^{-1}$ respectively.

The concentrations of Cd at the freshwater end was about 1.8 $ng \cdot kg^{-1}$. Within the low salinity (<7‰) region, concentrations of Cd and Ni increased rapidly with increasing salinity — an order of magnitude for Cd and double for Ni. This indicated that with increases in chlorinity and the amount of cations, such as Ca^{2+}, Mg^{2+}, and Na^{+}, parts of the particulate Cd and Ni transferred to the dissolved phase. At pH 7, the concentration of dissolved Ni was about 0.14 $\mu g \cdot kg^{-1}$, similar to that at the open sea end and with little variation in between. In the region with a salinity between 7 and 20‰, the concentration of dissolved Cd remained almost the same, indicating that particulate Cd was continuously transformed to the dissolved form. Beyond the region, where salinities were greater than 20 ‰, there was only a small amount of suspended particulates, and dissolved Cd also behaved conservatively, i.e.,

[3] $[\mathrm{Cd}]_{d,S>20} = -0.0021[S] + 0.084, (r = 0.90; n = 8).$

In low-salinity water, dissolved Fe decreased sharply with increasing salinity. At a salinity of 2.5‰, the concentration of Fe was only half that measured in the fresh water. The decrease was probably caused by the precipitation of colloidal Fe as

Table 3. Physicochemical parameters and dissolved metal concentration for surface seawater from Xiamen Harbour.

Area	Station	Salinity (‰)	Temperature (°C)	pH	Total suspended matter ($mg \cdot kg^{-1}$)	Dissolved metals ($\mu g \cdot kg^{-1}$)						
						Cu	Pb	Zn	Cd	Co	Ni	Fe
Northeast Harbour	1–2	29.88	29.9	8.18	3.6	0.36	0.008	0.036	0.015	0.005	0.13	0.14
West Harbour	3–8	29.32	28.9	8.31	7.0	0.48	0.014	0.10	0.025	0.011	0.15	0.16
Entry to Estuary	9–11	0.31	30.2	7.48	103	0.81	0.017	0.47	0.0018	0.008	0.081	0.96
Middle of Estuary	12–23	0.5–29	30.5	7.5–8.3	300–7	—	—	—	—	—	—	—
Outside Harbour	24–26	29.85	28.5	8.35	6.3	0.30	0.014	0.087	0.020	0.010	0.14	0.14
Reported in 1982	—	—	—	—	—	1.6±1.1	2.7±2.6	7.2±5.8	0.25±0.020	—	—	—

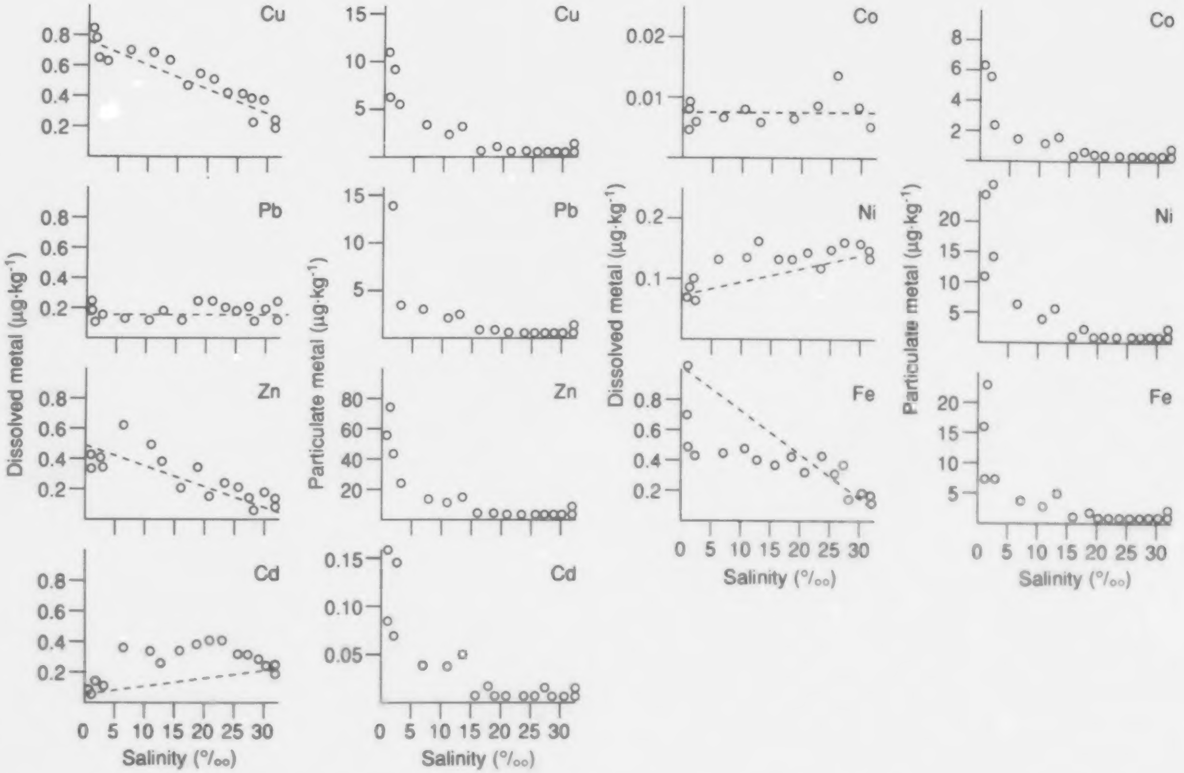

Fig. 2. Correlation of dissolved metals and particulate metals in Jiulongjiang Estuary with salinity. The dashed lines represent theoretical dilution trends.

electrolytes in the water increased. For salinities greater than 12‰ and a pH greater than 8.1, dissolved Fe also behaved conservatively, i.e.,

[4] $[\mathrm{Fe}]_{d,S>12} = -0.017[S] + 0.67$, ($r = 0.95$; $n = 9$).

Because speciation of trace metals between dissolved and particulate phases varied greatly within the estuarine area, metal fluxes to the open sea should be computed using their effective input concentrations rather than their concentrations in river water (Boyle et al. 1982; Edmond et al. 1985). In this report, linear equations depicting the conservative behaviour of dissolved metals were extrapolated to their respective origins to obtain effective input concentrations ($\mu g \cdot kg^{-1}$): Cd = 0.084, Co = 0.008, Cu = 0.79, Fe = 0.67, Ni = 0.14, Pb = 0.016, and Zn = 0.45.

Concentration and distribution of particulate metals

In the various regions of Xiamen Bay, amounts of suspended matter were fairly similar. A close relationship could be established between the concentration of a given particulate metal and the total suspended matter (TSM).

Figure 3 shows the relationship between the relative amount of particulate metal in the water body and TSM. When TSM ranged from 3 to 10 $mg \cdot kg^{-1}$, 90% of the particle-reactive elements, such as Co, Fe, and Pb (Santshi et al. 1980), and 70–90% of the Ni and Zn existed in their particulate forms; whereas most of the Cd and Cu existed in dissolved forms.

At the freshwater end of Jiulongjiang Estuary, TSM reached 300 $mg \cdot kg^{-1}$. This value decreased with increasing salinity, dropping to 3–6 $mg \cdot kg^{-1}$ in the open-sea area (Fig. 4). The decrease was probably caused by rapid flocculation and precipitation of particulates when predominantly negatively charged particles composed mainly of organic matter were mixed with strong electrolytic seawater. Changes in the concentration of particulate metals with salinity were similar to those of TSM with salinity (Fig. 2).

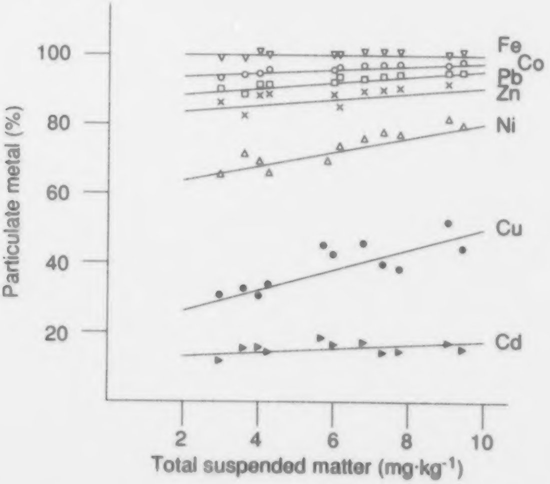

Fig. 3. Correlation between total suspended matter and the percentage of particulate metals in surface seawater from Xiamen Bay.

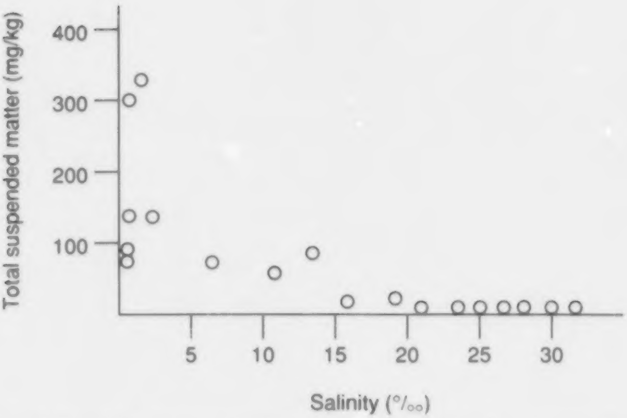

Fig. 4. Correlation between total suspended matter in surface seawater from Jiulongjiang Estuary.

Table 4 lists the relative amount of particulate metals observed at various salinities. In low-salinity water, 80% of the metals were in their particulate forms. At a salinity of 20‰, TSM was reduced to less than 5% of that in fresh water and there were some changes in the percentages of particulate metals. Now, Cd and Cu existed mainly in dissolved forms; there were slight decreases in the relative amount of particulate Ni and Zn; and 90% of Fe, Co, and Pb still existed in their particulate forms. Similar results were observed in high-salinity water.

As river water flows out to the coastal sea area, remaining particulate metals are precipitated further by various processes. Hence, the impact of particulate metals is limited to the estuarine and coastal areas, with only tiny amounts of these metals being carried to the open sea (Martin and Whitfield 1983).

Comparison between field and MEEE enclosure data

The utility of the experimental ecosystem enclosure depends on how realistically the enclosure simulates field conditions and how effectively results observed can be applied to the sea area under consideration. In this study, data from the MEEE control bag were compared with in-situ sampling data (Table 5). For

Table 4. Changes in relative amounts (%) of particulate metals with salinity in the estuarine area.

Salinity	Total suspended matter	% Particulate metal[a]						
(‰)	($mg \cdot kg^{-1}$)	Cu	Pb	Zn	Cd	Co	Ni	Fe
0.5	159	85	99	98	97	99	99	99.99
20	7.5	40	94	85	15	95	79	99.97
30	6.1	42	93	87	16	97	75	99.96

[a] % Particulate metal = $[M_p / (M_p + M_d)] \times 100$ where M_p = particulate metal and M_d = dissolved metal.

Table 5. Dissolved metal concentrations and partition coefficients (K_d)[a] in MEEE control bags and in Xiamen Harbour.

Source	Dissolved metal						
	Cu	Pb	Zn	Cd	Co	Ni	Fe
				Dissolved metals ($\mu g \cdot kg^{-1}$)			
Bag C1	0.41±0.06	0.022±0.008	0.20±0.05	0.032±0.004	0.019±0.011	0.25±0.07	0.22±0.11
Bag C2	0.42±0.04	0.017±0.007	0.24±0.12	0.036±0.006	0.020±0.011	0.25±0.06	0.20±0.10
Harbour[b]	0.35±0.10	0.017±0.009	0.10±0.05	0.022±0.003	0.008±0.002	0.14±0.02	0.14±0.02
				log K_d			
Bag C1	4.94	6.33	5.69	4.32	6.02	5.29	8.37
Bag C2	4.91	6.48	5.73	4.25	5.94	5.42	8.11
Harbour[b]	4.99	6.41	6.15	4.55	6.44	5.57	8.54

[a] K_d ($\mu g \cdot g^{-1}$ dry suspended matter)/($\mu g \cdot kg^{-1}$ seawater) × 10^3.

[b] Average of stations 6–8 and 24–26.

dissolved metals, concentrations measured in the enclosures and in the field were the same order of magnitude. MEEE values were slightly higher, but not more than 1.1 times the concentrations observed in the field. Concentrations in the two control bags varied by less than ±25%. These differences were probably caused by slight contamination during the experiment or as a result of sampling at different times.

Partition coefficients, K_d, in natural seawater and in the enclosures were the same magnitude, with slightly higher values being measured in the enclosures, probably because of higher concentrations of dissolved metals. In the controls, K_d for each metal decreased in the order Fe, Pb, Co, Zn, Ni, Cu, and Cd. As in the field survey, Cd and Cu existed mainly in their dissolved phases, whereas the other metals existed in their particulate phases.

Less than 2% of the total metal in the bag adheres to the bag wall (Li, J. et al. ("Pathways ..."), this volume). Thus, it is feasible to use MEEE enclosures to study the biogeochemistry of trace metals in coastal seawater. It should be possible to integrate the transfer model of metals and the dynamic transfer model in the enclosure to construct a transfer model of heavy metals applicable to field conditions.

Some limitations exist in applying enclosure results to the natural sea. For example, the hydrodynamics and conditions for water exchange in enclosures differ from those in the sea; the sediment–water interface is lacking in the enclosure; the plastic bag drifts easily; and observation time is limited. It is hoped that an improved enclosure will be designed in the near future to assist researchers in their studies of chemical oceanography.

Conclusions

In monitoring marine trace metals, the accuracy of the data depends on eliminating contamination during sampling and analysis.

Concentrations of dissolved and particulate metals (Cd, Co, Cu, Fe, Ni, Pb, and Zn) in surface water from Xiamen Bay were within the normal ranges of coastal seawater, but with the West Bay slightly polluted by heavy metals. Cd and Cu existed mainly in dissolved forms, whereas the other metals existed in their particulate forms. There was good correlation between the concentration of particulate metals and TSM.

When fresh water from Jiulongjiang River mixed with seawater, the conservative behaviour of dissolved Cu and Zn increased with increasing salinity. Concentrations of Co and Pb followed the simple conservative rule of mixing. In the cases of Cd, Fe, and Ni, their behaviour was nonconservative, i.e., concentrations of Cd and Ni increased in low-salinity water, and Fe transferred from the dissolved phase to the particulate phase. In high-salinity water, all of the metals behaved conservatively. More than 90% of the suspended particulates carried by the river were deposited in the estuary.

The MEEE enclosure is an excellent tool for studying the biogeochemistry of trace metals. Concentrations of dissolved metals within the enclosures differed from those from the field by less than 1.1 times, and relative deviations between values from the two enclosures were within ±25%.

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Boyle, E.A., Edmond, J.M. 1975. Copper in surface waters south of New Zealand. Nature, 253, 107–109.

Boyle, E.A., Huested, S.S., Jones, S.P. 1981. On the distribution of copper, nickel and cadmium in the surface waters of the North Atlantic and Pacific Ocean. Journal of Geophysical Research, 86 (C9), 8 048–8 066.

Boyle, E.A., Huested, S.S., Grant, B. 1982. The chemical mass balance of the Amazon plume: II — Copper, nickel and cadmium. Deep-Sea Research, 29, 1 355–1 364.

Bruland, K.W. 1980. Oceanographic distributions of cadmium, zinc, nickel and copper in the North Pacific. Earth and Planetary Science Letters, 47, 176–198.

Bruland, K.W., Franks, R.P. 1983. Mn, Ni, Cu, Zn and Cd in the western North Atlantic. *In* Wong, C.S., Boyle, E., Bruland, K.W., Burton, J.D., Goldberg, E.D., ed., Trace metals in sea water. Plenum Press, New York, NY, USA. 395–414.

Edmond, J.M., Spivack, A., Grant, B.C., Hu, M., Chen, Z., Chen, S., Zeng, X. 1985. Chemical dynamics of the Changjiang estuary. Continental Shelf Research, 4, 17–36.

Flegal, A.R., Patterson, C.C. 1983. Vertical concentration profiles of lead in the central Pacific at 15°N and 20°S. Earth and Planetary Science Letters, 64, 19–32.

Goldberg, E.D. 1965. Minor elements in sea water. *In* Riley, J.P., Skirrow, G., ed., Chemical oceanography, volume 1 (1st ed.). Academic Press, London, UK. 163–196.

Kanamorei, S. 1981. The species of trace elements in seawater. Chemical Sphere, 35 (4), 18–27. [In Japanese.]

Li, J., Zhen, Z., Du, R., Zhang, G. 1987. The concentrations and distribution of trace metals in the surface waters of Xiamen Harbour. Acta Oceanographica Sinica, 6, 380–388.

Magnusson, B., Westerland, S. 1983. Trace metal levels in sea water from the Skagerrak and the Kattegat. *In* Wong, C.S., Boyle, E., Bruland, K.W., Burton, J.D., Goldberg, E.D., ed., Trace metals in sea water. Plenum Press, New York, NY, USA. 467–474.

Martin, J.M., Whitfield, M. 1983. The significance of the river input of chemical elements to the ocean. *In* Wong, C.S., Boyle, E., Bruland, K.W., Burton, J.D., Goldberg, E.D., ed., Trace metals in sea water. Plenum Press, New York, NY, USA. 265–296.

Moore, R.M. 1978. The distribution of dissolved copper in the eastern Atlantic Ocean. Earth and Planetary Science Letters, 41, 461–468.

Riley, J.P., Skirrow, G., ed. 1975. Chemical oceanography, volume 1 (2nd ed.). Academic Press, London, UK.

Santshi, P.H., Li, Y-H., Carson, S. 1980. The fate of trace metals in Narragansett Bay, Rhode Island: radiotracer experiment in microcosms. Estuarine and Coastal Marine Science, 10, 635–654.

Sverdrup, J.U., Johnson, M.W., Fleming, R.H. 1942. The oceans: their physics, chemistry, and general biology. Prentice-Hall, Englewood Cliffs, NJ, USA.

Wong, C.S., Cretney, W.J., Piuze, J., Christensen, P., Berrang, P.G. 1977. Clean laboratory methods to achieve contaminant-free processing and determination of ultra-trace samples in marine environmental studies. National Bureau of Statistics, Special Publication 464.

Xu, Q., Huang, J., Yang, C., Huang, M., Chen, W., Lin, Y., Lin, L. 1986. Studies on the contents of metals and some other pollutants in sea water, sediments and organisms in the sea area near Xiamen Harbour. Marine Environmental Science, 5, 69–76. [In Chinese.]

Pathways and Fates of Heavy-Metal Mixtures in the Xiamen MEEE

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In 1985, the pathways and fates of two concentrations of heavy-metal mixtures in separate 10 m^3 bags were studied at Xiamen Bay. Initially, the dissolved metals were removed exponentially with the settling particles and their removal rate followed the order Pb > Hg > Zn > Cu > Cd. During a phytoplankton bloom, Zn was transferred biologically onto particles. Twenty-seven days after the experiment, most of the Cd and Cu remained in the dissolved phase, Hg and Pb were mainly transferred to the particles, and the amount of Zn was almost equal in both phases. The amount of metals retained on the wall of the bags was less than 2% of that in the bags. The association of heavy metals with zooplankton was rather unstable. Organic matter was found to be very important in the transfer of heavy metals.

Recently, the problem of heavy-metal pollution has received much attention. Some studies have been conducted to observe the toxic effects of heavy metals on marine organisms and the movement of metals in the marine environment. Over the last decade, the Marine Ecosystem Enclosure Experiment (MEEE) has been developed as a new tool to study the transfer and fate of marine pollutants (Topping and Windom 1977; Kremling et al. 1978; Santschi et al. 1980, 1983; Hunt et al. 1982; Santschi 1982; Wallace et al. 1982).

The coastal region of North America, where the Controlled Ecosystem Pollution Experiment (CEPEX) was conducted, is an area with deep water where biogeochemical cycles of elements are mainly regulated by the life cycle of planktonic organisms. In China, Xiamen Harbour is a relatively shallow and semienclosed bay. The seawater is affected by tidal action and the large amount of suspended matter carried down by the Jiulongjiang River. In this area, the effects of organic detritus and plankton on the transfer of elements may differ from those in North America. In 1985, scientists from Canada and China conducted a MEEE in Xiamen to study the transfer of heavy metals.

This report discusses the removal rates of two mixtures of heavy metals (Cd, Cu, Hg, Pb, and Zn) in low concentrations from the water column of the enclosures. It also discusses the effects of suspended particulate matter (SPM) and biological activities on transfer mechanisms and the fate of heavy trace metals.

Methods

Enclosures

Five cylindrical bags constructed from woven nylon and strengthened with polyethylene were used as the experimental enclosures. The bags, each 2 m in diameter and 3 m in depth, with the last 1 m in a conical shape, were mounted on a wooden raft placed in a pool (20 × 10 × 5 m) near the shore. The exchange rate of seawater between the pool and the harbour was 15 $m^3 \cdot h^{-1}$. At high tide, a diaphragm pump was used to fill the bags with seawater taken from 3 m depth 150 m offshore. The pool was shaded with a semiopaque roof to reduce the incident light intensity by about 50% (Wu, J., et al., this volume).

In each bag, polyethylene sediment traps (68 mm in diameter and 125 mm high), supported on a polypropylene rod and arranged at right angles, were placed at levels of 1 and 2 m below the surface. Several strips of bag material were hung at the end of the rod. A polyethylene cup (95 mm in diameter and 150 mm high) was suspended at the 3-m layer and used as another sediment trap (Fig. 1).

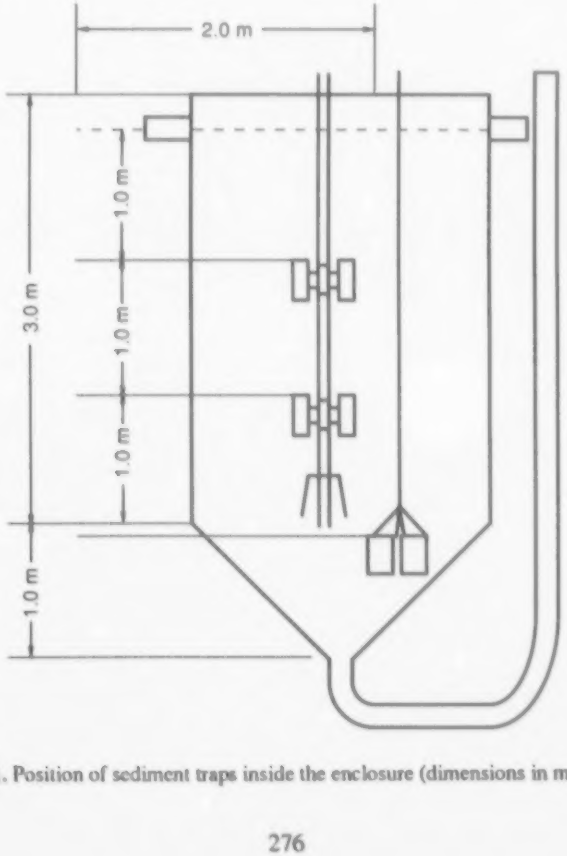

Fig. 1. Position of sediment traps inside the enclosure (dimensions in metres).

Metal treatments and sampling

Two bags (C1 and C2) were used as the control (no treatment), two bags (M1 and M2) were treated with a mixture of heavy metals in low concentrations, and the last bag (M3) was treated with a mixture of heavy metals in high concentrations. Background levels of metals in the control bags and concentrations of metals used in the treatments are listed in Table 1.

All of the bags were filled with about 10 m^3 of seawater before the experiment, which began on 18 April and ended on 15 May 1985. On day 0, additional nutrients (NO_3:SiO_4:PO_4 = 5:5:0.5 $\mu mol \cdot L^{-1}$) were mixed into the enclosures before seawater from the top 3 m was sampled using a peristaltic pump to determine background levels of various parameters. Appropriate metal mixtures were then added to the bags. Seawater and sediment samples were taken on days 1, 3, 5, 8, 11, 14, 17, 20, and 27. A strip of bag material was also removed periodically for analysis.

Sample analyses

Seawater samples were filtered through 0.4-µm Nuclepore filters under low vacuum. Material retained on the filters was washed with 5 mL of water, air dried at 50±2°C, and then weighed.

The filtrate was complexed with ammonium 1-pyrrolidine dithiocarbamate/ diethyl ammonium diethyl dithiocarbamate (APDC/DDDC), extracted with Freon-TF, back-extracted with dilute nitric acid, and then analyzed using a flameless atomic absorption spectrophotometer (PE-703) fitted with an HGA-500 graphite furnace (Li et al. 1986) for dissolved metals.

A subsample of the filtered material was agitated with 0.5 *N* HCl for 24 h and then analyzed using the flameless atomic absorption spectrophotometer for weakly bound particulate metals.

After extraction of the weakly bound metals, the residue was digested with a mixture of aqua regia and HF and analyzed using the flameless atomic absorption spectrophotometer for strongly bound particulate metals.

To determine total particulate metals, material retained on the filter was digested with aqua regia and HF and analyzed using the flameless atomic absorption spectrophotometer.

Data on Hg, particulate organic carbon (POC), particulate organic nitrogen (PON), chlorophyll *a*, and particle sizes are presented in the *MEEE-85 Xiamen Data Report* (MEEE Group 1985).

Chemical properties of the seawater in the bags during the experiment are listed in Table 2.

Table 1. Background and treatment levels ($\mu g \cdot kg^{-1}$) of heavy metals in the bags.

	Cd	Cu	Pb	Zn	Hg
C1, C2 background	0.03	0.38	0.02	0.25	0.002
M1, M2 treatment	1.0	3.5	0.3	3.5	0.2
M3 treatment	5.0	10.0	1.5	17.5	1.0

Table 2. Chemical properties of seawater in the experimental bags.

Parameter	Range
Surface sea temperature (°C)	17.6–23.5
Salinity (‰)	20.00–21.92
pH	8.11–8.54
$NO_2 + NO_3$ (µM)	25.4–0.1
NH_4 (µM)	0.43–9.5
PO_4 (µM)	0.94–0.00
$Si(OH)_4$ (µM)	56.5–21.0

Table 3. Half-removal time (d) of dissolved metals and suspended particles in the water column.

Bag	Cd	Cu	Pb	Zn	Hg	Particles
M1	89	21	5.4	13	6.7	3.4
M2	40	29	5.7	11	14.0	3.4
M3	30	16	5.8	22	9.0	3.9

Results and discussion

Because the experimental bags were supplemented with nutrients, growth of phytoplankton was fairly rapid. Phytoplankton bloomed from days 8 to 11 in bags C1, C2, M1, and M2. In bag M3, the bloom was delayed for 6 d. In this paper, the focus is on the transfer and fate of metal pollutants in the enclosures.

Removal of dissolved metals

Figure 2 shows changes in concentrations of dissolved metals with time. The curves illustrated can be divided into two time periods, the first 8 d, during which rapid changes in concentrations took place, and the last 20 d, during which changes, except for Zn, were relatively slow.

During the first 3 d of the experiment, concentrations of dissolved metals decreased rapidly. Trends, which were similar to the sinking of SPM (Fig. 2), could be described by first-order kinetic equations (Wallace et al. 1982). Calculated half-removal times of metals and SPM (Table 3) increased in the order SPM < Pb < Hg < Zn < Cu < Cd. Because Hg and Pb are particle-reactive elements (Santschi et al. 1980, 1983), their removal rates probably depended on the settling fluxes of particles in the water column. In contrast, Cd existed mainly in its dissolved phase, which only interacted with particulate matter through ion exchange or weak adsorption. It was less affected by sinking particulate matter, so its removal time was much longer.

During the last 20 d of the experiment, the total amount of SPM stayed almost the same. There was an obvious biological transfer of Zn during the bloom, indicating its bioreactivity. The relatively small changes in concentrations of Cd, Cu, Hg, and Pb indicated that biological transfer of these elements is either very slow or in an equilibrium state.

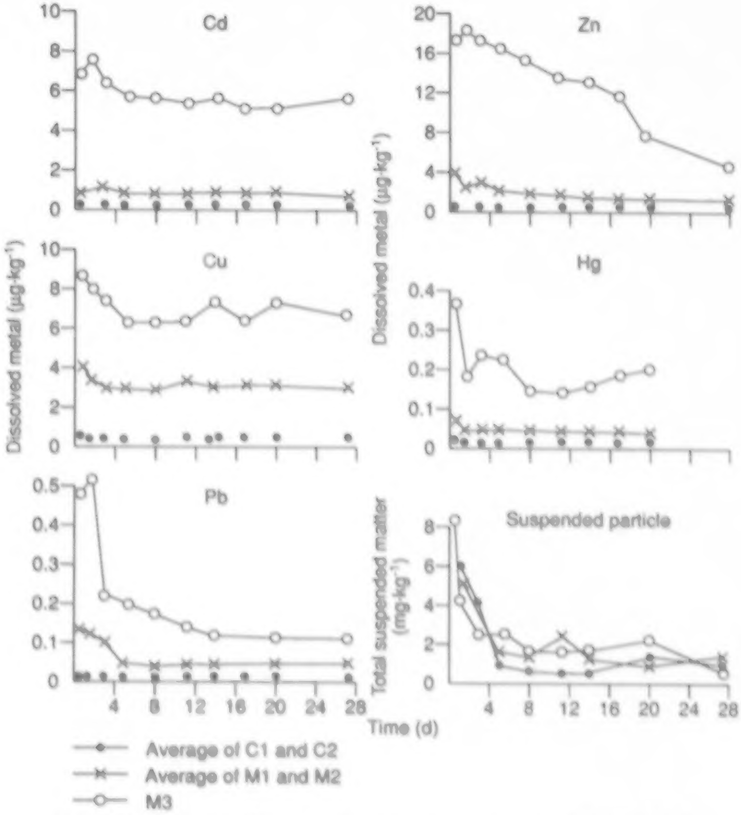

Fig. 2 (a). Changes with time in the concentrations of dissolved metal and total suspended matter (TSM).

Thus, when heavy metals were added in the enclosed ecosystems, they became involved in the physical, chemical, and biological aspects of transfer processes. The action of SPM adsorption, the activity of organisms, and the synergistic action of organisms all played an important role in removing metals from the water body.

Suspended particulate matter and particulate metals

Suspended particulate matter

Total SPM decreased exponentially from an initial concentration in the seawater of 8 mg·kg^{-1} to 1–2 mg·kg^{-1} near the end of the experiment. The decrease was accelerated both by the exclusion of turbulence and advection in the water in the bags and by the coagulation and sinking of particles during phytoplankton blooms. The addition of metals had no significant influence on the total amount of SPM.

Fluxes of sinking particles at the 3-m layer were calculated from the sediment trap data (Fig. 3). On day 1, the fluxes were the largest and were greater than

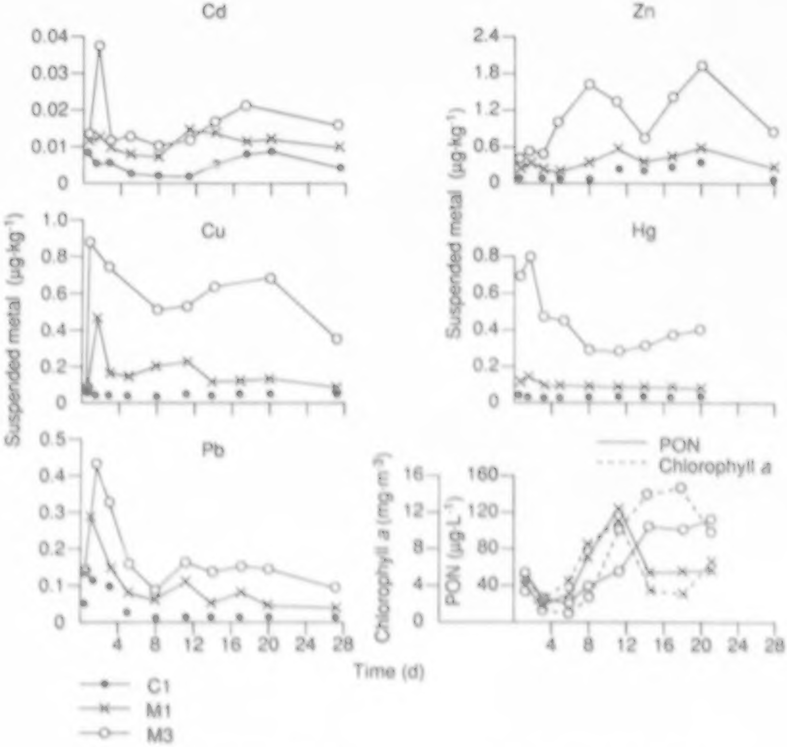

Fig. 2 (b). Changes with time in the concentrations of suspended metal, particulate organic nitrogen (PON), and chlorophyll *a*.

40 mg·cm^{-2}·d^{-1}. Thereafter, values decreased gradually, and 7 d later they fluctuated between 5 and 25 mg·cm^{-2}·d^{-1}.

The fluxes of particles among the 1-, 2-, and 3-m layers did not increase proportionally (Fig. 4). Maximum collection of particles occurred at the 1-m layer in which the density of plankton was probably the greatest among the three layers. At this layer, light attenuation was only about 50%, so photosynthesis was mainly carried out there. The next highest collection occurred at the 3-m layer, which was affected by resuspension of bottom sediment. From this layer, the amount of particles collected was far greater than the estimated loss of SPM. This indicated some authigenic production of particles in the bags. Other contributions included coagulation of fine particles (Lal 1980), deposition from the atmosphere, and resuspension of settled materials.

Values of *R*, i.e., the ratio between the amount collected from the sediment trap and the loss of particles from the water column, were also calculated. During the first 3 d, *R* values from all of the bags were in the range of 1.2±0.1. The value increased with the occurrence of resuspension, but its value was <1 during the bloom. Thus, *R* could be used as an index to investigate the mechanism of particle removal (Santschi et al. 1983).

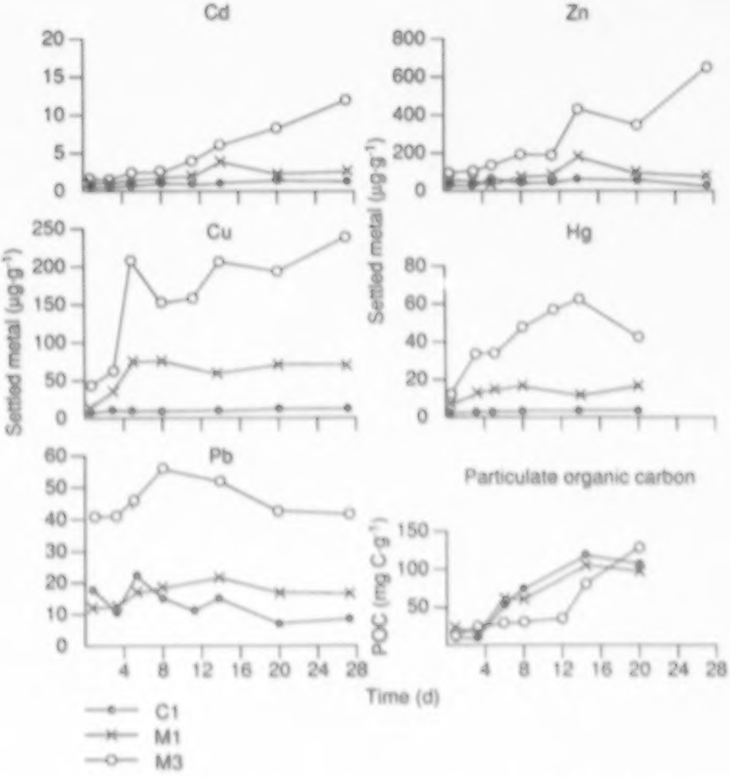

Fig. 2(c). Changes with time in the concentrations of settled metal and particulate organic carbon (POC).

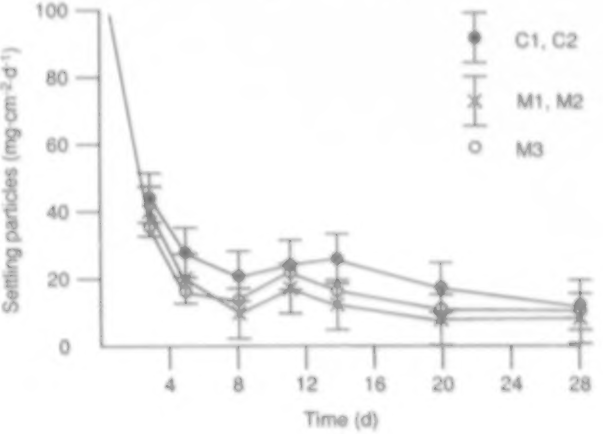

Fig. 3. Fluxes of settling particles at the 3-m layer.

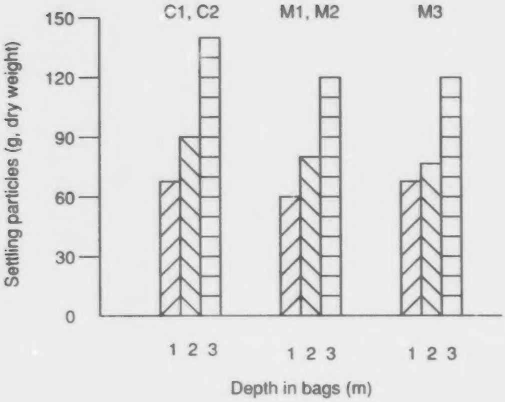

Fig. 4. Amounts of settling particles at the 1-, 2-, and 3-m layers.

Particles

Changes in the POC of suspended and settled materials were also measured to elucidate the effects of biological activity on the composition of particles (Fig. 5). It was found that the concentration of POC in suspended particles was twice the concentration measured in settled particles. During the early part of the experiment, the concentration of organic matter in the bags was relatively low and was basically within 2–5% of the total particulate dried weight. On day 11, the concentration of POC increased substantially in all of the bags. In the control and in bag M1, phytoplankton blooms were observed. The concentration of POC in the control, which increased to 22% of the total dried weight of the particles, was slightly higher than the concentration in bag M1. In bag M3, the bloom was delayed until day 20, at which time its POC concentration also increased. Suspended particles collected during the early part of the experiment were mainly composed of fine particles (<4 μm), but shifted toward larger particles (8–20 μm) in the latter part of the experiment (Fig. 6).

Concentrations of POC and PON at the end of the experiment were higher than those measured during the early part of the experiment (Fig. 7). Throughout the experiment, concentrations at the 1-m layer were consistently higher than those at the lower layers. During the experiment, the C:N ratio decreased from about 10:1 to about 6:1, similar to the ratio of healthy phytoplankton.

In summary, particulates in the bags shifted from inorganic fine particles during the early stages of the experiment to larger, mainly biogenic particles at the end of the experiment. Organisms in the upper layer were also more active than those in the lower layers.

Particulate metals

Figure 2 shows the variations in the concentrations of particulate (suspended) metals. In the bags treated with metals, concentrations reached their peak values early in the experiment, indicating adsorption of dissolved metals by particles. Concentrations decreased with the sinking of particles. For Zn, the bimodal curves

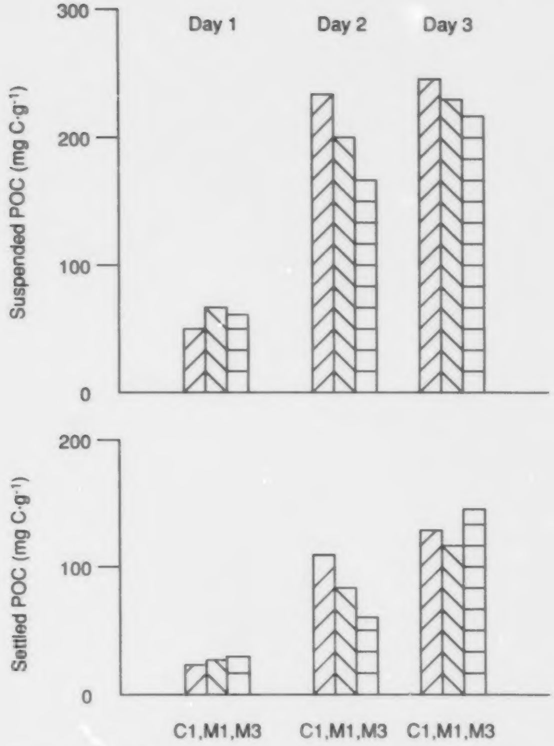

Fig. 5. Concentrations of particulate organic carbon (POC) in suspended particles and settling materials on days 1, 11, and 20.

suggest the uptake by organisms during the bloom correspond with decrease in the dissolved phase.

A partition coefficient, K_d, can be used to represent the degree of metal accumulation during the particulate phase. The K_d value for a given element reflects the particulate activity of the element. In bag M3, K_d values decreased in the order Pb > Hg > Zn > Cu > Cd (Fig. 8). This was the order observed for removal rates of dissolved metals.

During the first few days, the K_d of Hg increased rapidly, indicating that the added dissolved Hg was being transformed to the particulate phase. For Cd, the situation was reversed; most of the Cd remained in the dissolved phase in the water. The curve for Zn shows three different slopes, representing the early increase in the dissolved phase, the biological transfer, and the particulate adsorption and sinking phase.

After the addition of metals, large portions of the metals were adsorbed onto settling inorganic and organic particles. The amounts settled increased with the amounts of metals added. As the POC content increased, there were corresponding

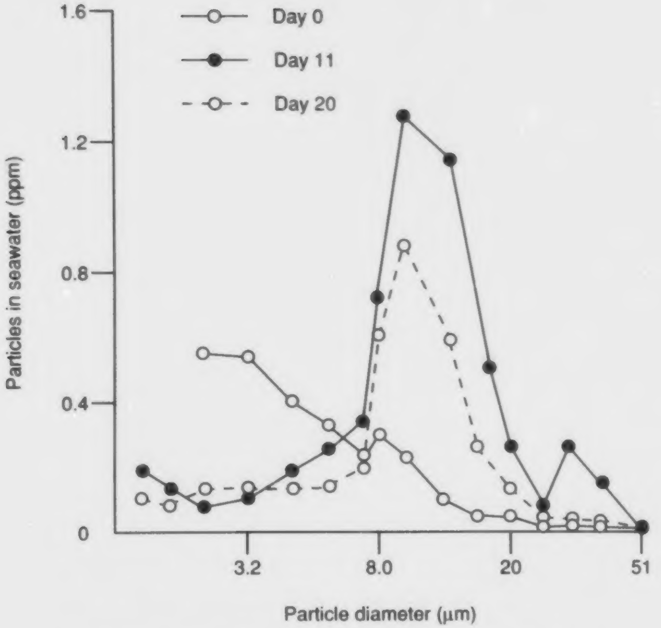

Fig. 6. Particle-size distribution of suspended particulate matter in bag M3 on days 1, 11, and 20.

increases in the concentrations of settled Cd, Cu, Hg, and Zn (Fig. 2), indicating that organic carbon was able to undergo complexing with these metals.

Some interesting results were obtained by comparing the concentrations of metals in suspended matter and settling materials collected from the three layers (Fig. 9). The concentrations of Cd, Zn, and Pb decreased in the order suspended matter, 1-m, 2-m, and 3-m settling materials, which was the same order as the decreases of POC. However, the concentration of Cu increased in the same order. These variations were probably the result of different biogeochemical processes involving the metals in the enclosures. Some of the Cd, Pb, and Zn was released to the water body with the weakly bound ligands following the death and decay of planktonic organisms. In the case of Cu, it accumulated on settled materials as organic matter decomposed during remineralization. Organic matter was the main factor controlling the distribution of trace metals in the enclosures. It could enhance the solubility of metals in water or the partition of trace metals on particles (Kuiper 1982; Morel et al. 1983; Hong 1984).

Budget of metal in the enclosures

Table 4 lists the amounts of the various metals in their respective phases during the experiment. Some of the results are summarized below.

- The metals adsorbed on the bag wall amounted to less than 2% of those in the water.

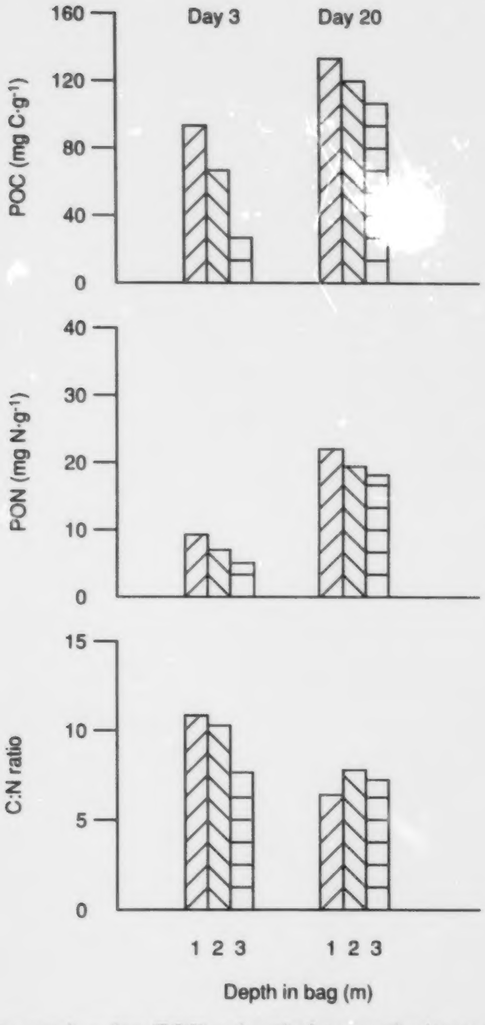

Fig. 7. Particulate organic carbon (POC) and particulate organic nitrogen (PON) in settling materials from depths of 1, 2, and 3 m in bag M1 on days 3 and 20.

- In the two control bags, total amounts of each metal on day 27 were higher than those present on day 0. The proportion of the increase varied among the different metals, but was highest in the cases of Pb and Zn. These increases indicated the existence of some other sources of heavy metals, such as contamination or atmospheric deposition during the experiment.

- During the experiment, bag M2 slanted considerably, which increased resuspension. Most of the data for bag M2, especially total amounts of metals recovered, were not acceptable. In bags M1 and M3, recovery rates

Table 4. Budget of metals (mg per bag).

	Cd		Cu		Pb		Zn		Hg	
	Day 0	Day 27	Day 0	Day 27	Day 0	Day 27	Day 0	Day 27	Day 0	Day 20
Bag C1										
Dissolved	0.30	0.34	3.7	4.5	0.13	0.18	2.3	2.5	0.021	0.031
Particulate	0.08	0.03	0.5	0.07	0.69	0.15	3.7	0.4	0.012	0.001
Added	—	—	—	—	—	—	—	—	—	—
On wall	—	0.01	—	0.14	—	0.04	—	0.1	—	—
Settled	—	0.14	—	1.75	—	2.1	—	9.1	—	0.028
Total	0.38	0.52	4.2	6.5	0.82	2.5	6.0	12.1	0.033	0.060
Bag C2										
Dissolved	0.36	0.39	3.9	4.7	0.13	0.11	2.4	2.5	0.035	0.030
Particulate	0.14	0.02	0.5	0.14	0.53	0.16	3.6	0.7	0.023	0.003
Added	—	—	—	—	—	—	—	—	—	—
On wall	—	0.01	—	0.13	—	0.05	—	0.1	—	—
Settled	—	0.10	—	1.45	—	1.9	—	7.1	—	0.029
Total	0.50	0.52	4.4	6.4	0.66	2.2	6.0	10.4	0.058	0.062
Bag M1										
Dissolved	0.35	8.4	3.7	23.4	0.12	0.45	2.7	9.3	0.053	0.27
Particulate	0.12	0.10	0.5	0.6	0.75	0.24	3.8	2.5	0.000	0.32
Added	10	—	35	—	3.0	—	35	—	2.0	—
On wall	—	0.03	—	0.3	—	0.08	—	0.2	—	—
Settled	—	0.24	—	4.1	—	2.4	—	18.0	—	0.93
Total	10.5	8.8	39.2	28.4	3.9	3.2	41.5	30.0	2.1	1.5

Bag M2										
Dissolved	0.29	11.9	3.5	33.9	0.12	0.42	2.8	9.8	0.031	0.43
Particulate	0.13	0.14	0.6	1.6	0.78	0.35	3.8	3.0	0.006	0.56
Added	10	—	35	—	3.0	—	35	—	2.0	—
On wall	—	0.03	—	0.4	—	0.09	—	0.2	—	—
Settled	—	0.25	—	9.1	—	2.9	—	26.1	—	2.38
Total	10.4	12.3	39.1	45.0	3.9	3.8	41.6	39.1	2.0	3.4
Bag M3										
Dissolved	0.28	52	3.1	67.3	0.14	1.05	2.3 45	0.037	2.0	—
Particulate	0.13	0.15	0.5	3.7	0.64	0.90	2.7 10	0.001	2.6	—
Added	50	—	100	—	15	—	175	—	10	—
On wall	—	0.04	—	1.4	—	0.14	—	1.4	—	—
Settled	—	0.57	—	18.0	—	5.6	—	42	—	4.9
Total	50.4	52.8	103.6	90.4	15.8	7.7	180	98.4	10	9.5

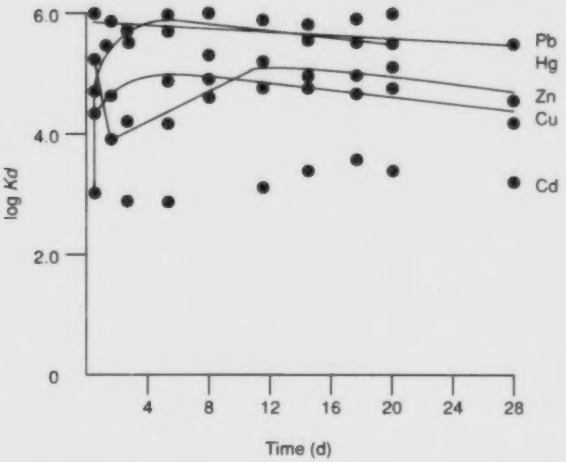

Fig. 8. Changes in the partition coefficient (K_d) of metals between solid and liquid phases in bag M3 with time. K_d = [particulate metal (μg·g^{-1} dry weight)/dissolved metal (μg·kg^{-1})] × 10^3.

fluctuated between 50 and 90%. Incomplete recovery was probably caused by leaking of the enclosures, changes in water volume, evaporative loss of metals, or errors introduced during sampling and analysis.

- More than 80% of the Cd and 60% of the Cu remained in dissolved phases. More than 60% of the Pb and 50% of the Hg transferred to settled materials. For Zn, amounts were equally split between the two phases.

Heavy metals in zooplankton

Table 5 lists the concentrations of various heavy metals and their concentration factors (K_d) in zooplankton at the end of the experiment. Cd, Cu, Pb, and Zn added during the experiment have now been transferred to zooplankton. A high concentrations of dissolved metals always corresponded with small K_d values. Amounts of Cd, Cu, and Zn in zooplankton were higher than their respective concentrations in the particles before treatment, whereas amounts of Pb in zooplankton and initial suspended particulate matter were about the same in M1 and M2.

Bonds between heavy metals and zooplankton were very weak. Most of these metals could be extracted with dilute acid (Table 6). The percentages of weak-binding metals decreased in the order Cd, Zn, Cu, and Pb, and they also decreased in the order zooplankton, SPM, and settling particles. In an enclosed ecosystem, only a very small proportion of each heavy metal was removed by zooplankton.

Conclusions

During the first few days of the experiment, the concentration of dissolved metals decreased exponentially together with the original particles in the bags.

Table 5. Concentration of heavy metals and their concentration factor (K_d)[a] in zooplankton.

Bag	Cd		Cu		Pb		Zn		Co		Ni	
	ppm	K_d	ppm	K_d	ppm	K_d	ppm	K_d	ppm	K_d	ppm	K_d
C1, C2	4.5	1.5×10^5	29	6.3×10^4	31	2.1×10^6	264	1.1×10^6	5.6	4.3×10^5	13	4.5×10^4
M1, M2	7.9	7.9×10^3	105	3.7×10^4	36	8.2×10^5	293	3.3×10^5	6.1	—	11	—
M3	21	4.0×10^3	118	1.8×10^4	68	6.4×10^5	353	7.8×10^4	6.2	—	13	—
Initial SPM[b]	0.64	2.1×10^4	32	9.1×10^4	39	2.8×10^6	112	4.7×10^5	29	2.9×10^5	71	2.3×10^5
Ocean plankton[c]	0.19–54		3–26				21–400				1–25	

[a] K_d = [metal content in zooplankton ($\mu g \cdot g^{-1}$ dry weight)] / [dissolved metal concentration in seawater ($\mu g \cdot kg^{-1}$)] × 10^3.

[b] SPM = Suspended particulate matter.

[c] From Collier and Edmond (1984).

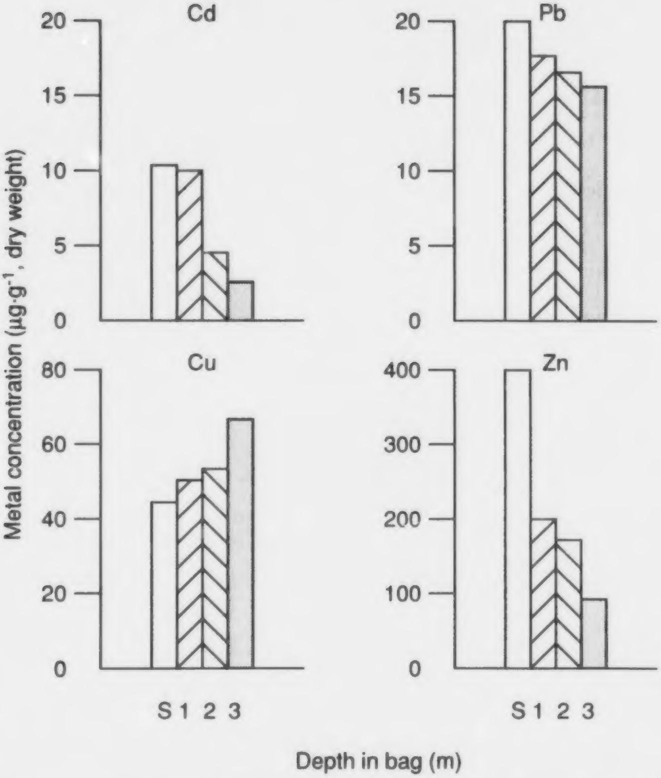

Fig. 9. Concentrations of metals in suspended particles (S) at depths of 0–3 m and settling materials at depths of 1, 2, and 3 m in bag M1 at the end of the experiment.

Table 6. Percentage of weak-binding metals in various types of particles at the end of the experiment.

Particle	Bag	Cd	Cu	Pb	Zn
Zooplankton	C1	87	72	42	75
	M1	95	66	55	81
	M3	96	93	75	93
Suspended particles	C1	65	54	31	60
	M1	86	66	45	72
	M3	85	76	67	70
Settled particles	C1	76	49	27	53
	M1	80	53	34	63
	M3	39	53	54	60

Removal rates decreased in the order Pb, Hg, Zn, Cu, and Cd. After the bloom, the concentration of Zn continued to decrease, whereas concentrations of Cd, Cu, Hg, and Pb changed little. On day 27, more than 80% of the Cd and 60% of the Cu remained in dissolved phases, and more than 60% of the Pb and 50% of the Hg transferred to settled materials. For Zn, the partition was about the same between the two phases.

Organic matter was an important factor in the partition of heavy metals between particulate and dissolved phases. Different trends in the concentrations of Cd, Cu, Pb, and Zn among suspended matter and settling materials from the three collection layers indicated the complexity of biogeochemical cycles of heavy metals in a marine environment.

Acknowledgments

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Collier, R., Edmond, J. 1984. The trace element geochemistry of marine biogenic particulate matter. Progress in Oceanography, 13, 113–199.

Hong, H.S. 1984. Characteristics of metal-particle association in MERL mesocosm experiments. University of Rhode Island, Kingston, RI, USA. PhD thesis. 27 pp.

Hunt, C.D., et al. 1982. Controlled marine ecosystems — a tool for studying stable trace metal cycles: long-term response and variability. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 111-112.

Kremling, K., Pivze, J., Von Brockel, K., Wong, C.S. 1978. Studies on the pathways and effects of cadmium in controlled ecosystem enclosures. Marine Biology, 48, 1–10.

Kuiper, J. 1982. Ecotoxicological experiments with marine plankton communities in plastic bags. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 181–193.

Lal, D. 1980. Comments on some aspects of particulate transport in the ocean. Earth and Planetary Science Letters, 49, 520–527.

Li, J., Johnson, W.K., Wong, C.S. 1986. APDC/DDDC Freon extraction for flameless atomic absorptiometric determination of trace metals in seawater. Environmental Chemistry, 7, 75–79. [In Chinese.]

MEEE Group. 1985. Xiamen data report 1985. Third Institute of Oceanography, Xiamen, People's Republic of China. Unpublished, 109 pp.

Morel, F.M.M., et al. 1983. Trace metals and plankton in the oceans: facts and speculations. *In* Wong, C.S., Boyle, E., Bruland, K.W., Burton, J.D., Goldberg, E.D., ed., Trace metals in seawater. Plenum Press, New York, NY, USA. 63–80.

Santschi, P.H. 1982. Application of enclosures to the study of ocean chemistry. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 63–80.

Santschi, P.H., Li, Y.-H., Carson, S.R. 1980. The fate of trace metals in Narragansett Bay, Rhode Island: radiotracer experiments in microcosms. Estuarine and Coastal Marine Science, 10, 635–654.

Santschi, P.H., Adler, D.M., Amdurer, M. 1983. The fate of particles and particle-reactive trace metals in coastal waters: radioisotope studies in microcosms. *In* Wong, C.S., Boyle, E., Bruland, K.W., Burton, J.D., Goldberg, E.D., ed., Trace metals in seawater. Plenum Press, New York, NY, USA. 331-349.

Topping, G., Windom, H.L. 1977. Biological transport of copper at Loch Ewe and Saanich Inlet: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 135–141.

Wallace, G.T., Jr., Seibert, D.L., Holzknect, S.M. 1982. The biogeochemical fate and toxicity of Hg in controlled experimental ecosystems. Estuarine and Coastal Shelf Science, 15, 151–182.

Biogeochemical Behaviours of Heavy Metals in Marine Enclosed Ecosystems

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The biogeochemical processes of heavy metals depend on the chemical nature of the elements. Zinc is bioactive and during a phytoplankton bloom, the amount of particulate Zn increases proportionately with the concentration of chlorophyll a. Mercury shows an affinity for particulates. Most of the Hg is in the particulate phase, but the amount of Hg does not increase proportionately with the occurrence of phytoplankton blooms. Copper and cadmium tend to remain in solution, so their amounts in the particulate phase do not depend on the abundance of phytoplankton. This report discusses the removal rate and removal half-life of various heavy metals in the water column. The removal rate depended on the element, the abundance of phytoplankton, and the amount of settling particles. Chemical speciation of heavy metals is also discussed and concentrations of heavy metals in settled materials are determined. The amount of ^{65}Zn in settled materials increased with the occurrence of phytoplankton, whereas particulate Cd, Cu, and Hg did not vary with phytoplankton abundance. There was some dissolution of Cu and Hg in bags treated with sediment. Suppression of primary productivity in these bags was probably caused by particulates, which attenuate light.

Early experiments using marine enclosed ecosystems were mainly conducted by biological oceanographers. For the last decade, many chemical oceanographers have also used this new tool to study the transfer and fate of pollutants. These include the controlled experimental ecosystems (CEEs) used by Wallace et al. (1982) in Canada, the plastic bags used by Kuiper (1982) in the Netherlands, the Loch Ewe closed systems used by Topping et al. (1982) in the UK, the systems used by Santschi (1982) at the Marine Ecosystems Research Laboratory (MERL) in the USA, and the sediment seawater enclosures and portable multiple enclosure system used Wong et al. (this volume) in Canada.

Environmental conditions affecting an enclosed experiment lie between the enormous variability of the real world and the much simpler laboratory experiment. For investigating the biogeochemistry of elements, an enclosed ecosystem can be used to isolate relevant natural processes. For example, in estuarine ecosystems, sources of particulate organic matter include photosynthesis of phytoplankton as well as terrigenous organic matter transported by the rivers or deposited from the atmosphere. This variety of sources creates further difficulties in the study and quantitative estimation of multiple natural processes.

In an enclosed ecosystem experiment, photosynthesis of plankton, with the concurrent loss of nutrients, can be regarded as the sole source of particulate organic carbon (POC) or particulate organic nitrogen (PON). Moreover, material that settles to the bottom of the bag can be removed periodically to prevent resuspension of this material by water movement and the release and transformation of any substance during diagenesis.

This report describes the results of studies on the biogeochemistry of heavy metals conducted during the 1985 Marine Ecosystem Enclosure Experiment (MEEE) in Xiamen, People's Republic of China.

Methods

Experimental enclosure

The experiment was conducted between 18 April and 8 May 1985 in the experimental pool located at the Third Institute of Oceanography, Xiamen. Details of the experimental design and sampling methods are given elsewhere in this volume (Wu, J., et al., this volume). Briefly, the experimental bags (2 m in diameter by 4.5 m deep, with a volume of 10 m^3) were made of polyethylene. The bags were filled with seawater pumped from 150 m offshore during high tide. Nutrients were added to the enclosures before the experiment. The salinity of the seawater was about 20‰ and the temperature was about 20°C.

Heavy-metal treatment

Nine bags were used in this experiment. The control bags were designated C1 and C2. Bags treated with metals were designated M1, M2, and M3. After the addition of heavy metals, the concentrations of metals in bags M1 and M2 were about 10 times those measured in the seawater of Xiamen Bay, i.e., 1.0 $\mu g\ Cd/\cdot kg^{-1}$, 3.5 $\mu g\ Cu\cdot kg^{-1}$, and 0.2 $\mu g\ Hg\cdot kg^{-1}$, whereas the concentrations of metals in bag M3 were about 50 times those found in natural seawater, i.e., 5.0 $\mu g\ Cd\cdot kg^{-1}$, 10.0 $\mu g\ Cu\cdot kg^{-1}$, and 1.0 $\mu g\ Hg\cdot kg^{-1}$. Three other bags, designated S1, S2, and S3, were treated with various amounts of sediment. After the addition of sediment, the concentration of sediment in bags S1 and S2 was about 11 ppm, whereas the concentration of sediment in bag S3 was about 110 ppm. Another bag was treated with ^{65}Zn with an initial concentration of $10^{-8}\ Ci\cdot L^{-1}$ (370 $Bq\cdot L^{-1}$) in the seawater. Two other metals, Pb and Zn, which were also used in the bags treated with metal, are discussed by Li, J., et al. ("Distribution ..." this volume).

Sampling

Seawater samples were integrally pumped from the upper 3 m of the water columns and stored in polyethylene bags that had been pretreated with acid and washed. Subsamples of the collected seawater were then taken and used for various measurements. Sediment was collected in a sediment trap placed on the bottom of each bag.

Analysis

Seawater samples were filtered through a 0.45-μm membrane filter. Concentrations of dissolved Cd and Cu were determined by flameless atomic absorption spectrophotometry (GFAAS) and anodic stripping voltammetry (ASV); the concentration of Hg was determined by cold vapour atomic absorptiometry.

Particles retained on the filter were processed to determine concentrations of particulate heavy metals. Cadmium and copper on the particles were extracted using 0.5 *N* HCl; Hg was then obtained by a complete digestion of the particles. Analytical determinations followed the methods described previously.

Soluble Cd and Cu in settled materials were extracted using 0.5 *N* HCl and Hg was obtained by complete digestion. Amounts of ^{65}Zn in unfiltered water, filtered water, and settled materials were determined by gamma-ray spectrometry.

The concentration of chlorophyll *a* was determined by in-vitro fluorometry and the concentration of particulate organic carbon was determined using a CHN analyzer.

Results and discussion

Chlorophyll *a* and particulate organic carbon

Figure 1 shows variations in the concentration of chlorophyll *a* over the course of the experiment. In bags M1 and M2, the timing of phytoplankton growth was similar. Before the phytoplankton bloom (day 0–day 5), the concentration of chlorophyll *a* was very low. During the bloom (day 5–day 11), there was a rapid increase in the concentration of chlorophyll *a*. This was followed by a recession period from day 11 to day 17 and possibly the start of another bloom between days 17 and 20. The periodicity of plankton blooms in bags M1 and M2 resembled that observed in the control bags. In bag M3, the phytoplankton bloom was delayed by 6 d.

In the controls, the concentration of POC between days 6 and 12 was higher than the initial concentration (Fig. 2). Peak values occurred around day 8, coincident with rapid phytoplankton growth. During this period, there was also a rapid decrease in inorganic nutrients, such as nitrate and phosphate. In the two bags treated with a mixture of heavy metals in low concentrations, peak values were comparable with those in the controls, but were delayed slightly in time; in the bag treated with a mixture of heavy metals in high concentrations, the peak was delayed for at least 6 d and its value was significantly reduced. These results suggest that

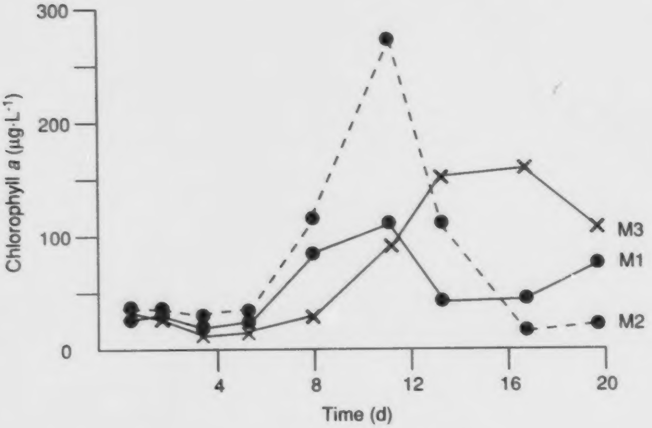

Fig. 1. Variation of chlorophyll *a* with time.

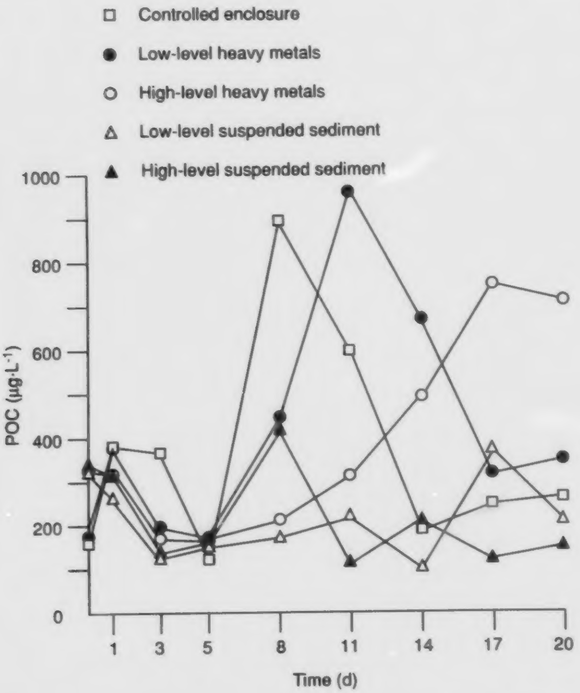

Fig. 2. Concentrations of particulate organic carbon (POC).

primary productivity in the ecosystem was not affected by heavy metals in low concentrations, but that it was definitely suppressed by heavy metals in high concentrations.

Suspended particles

The initial concentration of suspended particles in bags C1 and M1 (Fig. 3) was about 8 mg·L^{-1}. In the intertidal zone, terrigenous material was kept in suspension by rough sea conditions on the day seawater was pumped into the bags. Concentrations decreased sharply during the first 3 d and then remained at about 1–2 mg·L^{-1} thereafter. Before the phytoplankton bloom, suspended particles consisted mainly of terrigenous matter, which sank rapidly to the bottom. During the bloom, suspended particles were composed mainly of organic matter.

Removal rate and half-life of heavy metals in the water column

The removal of a given heavy metal depended on the nature of the element, the growth of phytoplankton, and the amount of settled materials. Because Hg had a high affinity for particles, its removal was controlled by particulates (Fig. 3). Before the phytoplankton bloom, there was a large amount of suspended material in the water column. This was composed mostly of fine terrigenous particles with large relative surface areas, which would have a high adsorption capacity for Hg. Thus, Hg was removed rapidly during the early stage (Fig. 4). During the bloom, there was an increase in the relative amount of organic carbons in suspended particles, which were chiefly the biogenic products of plankton. Particle size depended mainly on the activity of the bloom, i.e., the larger the bloom, the larger the particles and, therefore, the smaller the relative surface area. Thus, adsorption for Hg and its removal decreased.

The bioactive element Zn was removed during the early stage by adsorbing onto settling particles (Fig. 5). However, its affinity for particulates was not as strong as that of Hg, so its removal rate was lower than that of Hg. During the phytoplankton

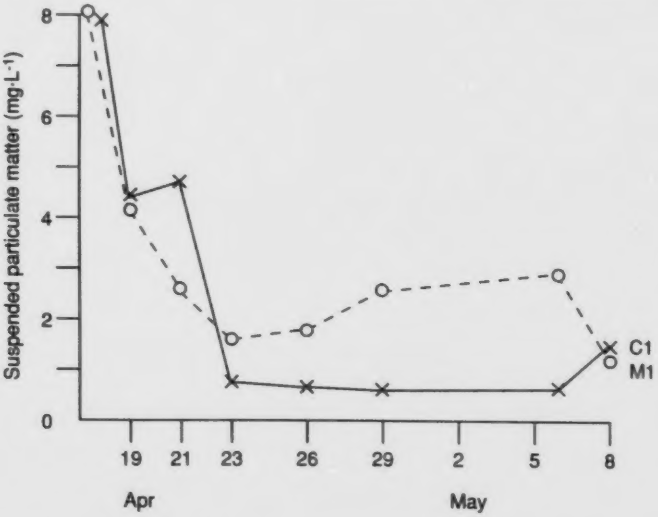

Fig. 3. Concentration of suspended particulate matter over time.

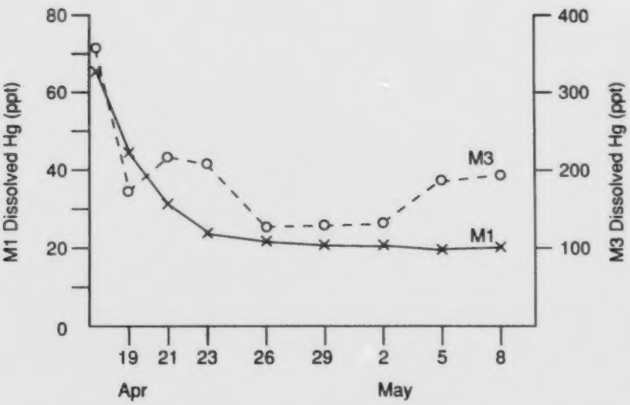

Fig. 4. Concentrations of dissolved Hg.

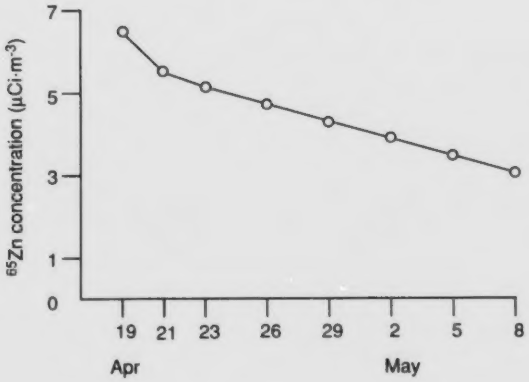

Fig. 5. Concentrations of ^{65}Zn (note: 1 Ci = 37 GBq).

bloom, when most terrigenous particles had already settled, Zn was adsorbed onto biotic suspended particles and was also being incorporated into the body of the organisms. Hence, the amount of particulate Zn increased significantly and the removal rate of Zn did not decrease as for the other elements.

Cadmium had a high affinity for the dissolved phase. Chemical processes between Cd and particulates consisted mainly of ion exchange and weak adsorption; its removal rate, therefore, was about the same before and after the phytoplankton bloom (Fig. 6). The behaviour of Cu was somewhere between that of Hg and Cd; thus, its removal rate was fairly rapid during the prebloom period and slowed down after the bloom (Fig. 7).

Table 1 lists the removal half-life of the various metals during the experiment, disregarding the separate periods of the bloom. All concentrations (C) were graphed against time (t) as $\ln C = bt + a$ where a and b are constants, and the respective removal half-life was computed as $T_{0.5} = (\ln 2/-b)$. Thus, in bag M3, the

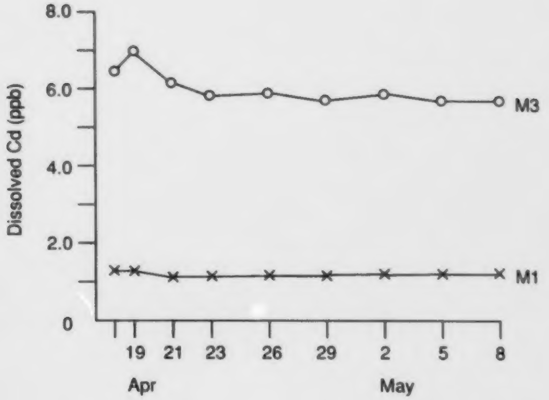

Fig. 6. Concentrations of dissolved Cd.

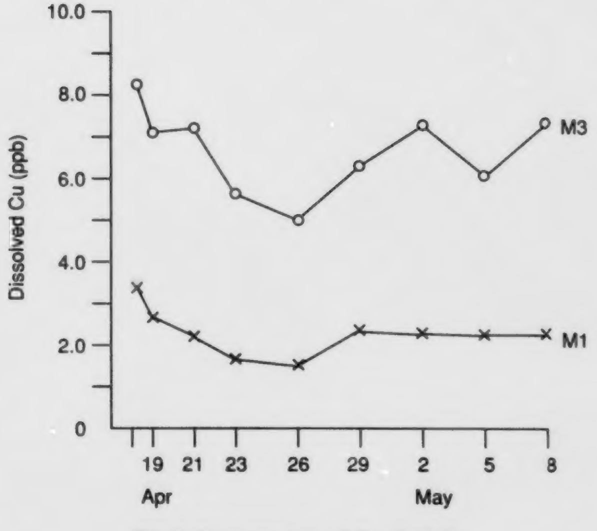

Fig. 7. Concentrations of dissolved Cu.

removal half-lives for Cu and Hg were 23 and 15 d respectively. In the radioisotope bag, the removal half-life of ^{65}Zn was 13.2 d.

Chemical speciation of heavy metals

Table 2 lists the concentrations of the various metals in the dissolved and particulate phases at the end of the experiment. In the control bags, about 86% of the Cd was in the dissolved phase and the rest was in the HCl-extractable particulate phase; 93% of the Cu was in the dissolved phase and 7% was in the particulate phase; and 76% of the Hg was in the dissolved phase and 24% was in the particulate phase. After the addition of heavy metals, dissolved Cd, Cu, and Hg constituted

Table 1. Half-life of the particulate and dissolved phases of heavy metals in the water column of different enclosures.

Metal	Phase	Regression equation	Date	Relative parameter (n)	Half-life (d)
Bag M3					
Cu	Dissolved	$\ln C = 11.1 - 0.020t$	18 Apr–5 May	-0.93 (8)	23
	Particulate	$\ln C = 9.6 - 0.10t$	18–29 Apr	-0.88 (5)	6.9
Cd	Dissolved[a]				
	Particulate	$\ln C = 5.4 - 0.11t$	19–29 Apr	-0.75 (4)	6.3
Hg	Labile	$\ln C = 7.8 - 0.045t$	18 Apr–2 May	-0.70 (7)	15
	Particulate	$\ln C = 8.8 - 0.046t$	19 Apr–8 May	-0.90 (8)	15
Bag S1, S2					
Cu	Particulate	$\ln C = 8.4 - 0.20t$	19 Apr–8 May	-0.84 (8)	3.5
Cd	Particulate	$\ln C = 4.2 - 0.031t$	18 Apr–8 May	-0.51 (8)	22
Hg	Particulate	$\ln C = 6.6 - 0.038t$	18 Apr–8 May	-0.82 (9)	1.8
Bag S3					
Cu	Particulate	$\ln C = 9.3 - 0.18t$	19 Apr–8 May	-0.84 (7)	3.9
Cd	Particulate	$\ln C = 4.6 - 0.055t$	19 Apr–8 May	-0.75 (7)	13
Hg	Particulate	$\ln C = 8.9 - 0.49t$	18 Apr–8 May	-0.87 (9)	1.4
Bag M2					
Hg	Labile	$\ln C = 6.5 - 0.030t$	18 Apr–8 May	-0.82 (9)	23
	Particulate	$\ln C = 7.5 - 0.060t$	18 Apr–8 May	-0.97 (9)	12
Bag M1					
Hg	Labile	$\ln C = 6.1 - 0.065t$	18 Apr–5 May	-0.87 (8)	11
	Particulate	$\ln C = 7.0 - 0.058t$	18 Apr–8 May	-0.94 (9)	12

Note: The concentration unit is $\mu g \cdot (10\ m^3)^{-1}$. Hg was determined by cold vapour atomic absorptiometry. Cd and Cu were determined by flameless atomic absorption spectrophotometry except dissolved Cd and Cu in bag M3 which were determined by anodic stripping voltammetry.

[a] Dissolved cadmium concentration remained relatively constant ($4.31 \times 10^4 \pm 0.2 \times 10^4$; $n = 9$) over the duration of the experiment.

99, 90, and 32% of the total Cd, Cu, and Hg, respectively; the remainder was mainly in the respective particulate phases. Because Hg had a high affinity for particles, the Hg added during the experiment was quickly transferred onto the particles, thereby raising the proportion of particulate Hg. Conversely, Cd tended to remain in solution and was transferred from the particles to the seawater.

Table 2 also indicates that during the bloom period, there were no significant increases in the proportion of particulate Cd, Cu, and Hg.

Relationship between the relative amounts of particulate metal and chlorophyll *a*

Two parameters that characterize the biology of a marine ecosystem are chlorophyll *a* and primary production. Of the two, chlorophyll *a* should have a better

Table 2. Concentrations (μg·(10 m^{-3})) of Cd, Cu, and Hg in particulate and dissolved phases.

Metal	Phase	April							May			%
		18[a]	18	19	21	23	26	29	2	5	8	
Bag M3												
Cd	Dissolved	2.8×10^{2}	6.8×10^{4}	7.2×10^{4}	6.2×10^{4}	5.9×10^{4}	5.8×10^{4}	5.5×10^{4}	5.7×10^{4}	5.3×10^{4}	5.3×10^{4}	9.6±0.1
	Particulate	1.4×10^{2}	1.6×10^{2}	3.6×10^{2}	1.1×10^{2}	1.1×10^{2}	—	85	—	—	2.0×10^{3}	0.4±0.1
Cu	Dissolved	3.1×10^{3}	8.5×10^{4}	7.1×10^{4}	7.1×10^{4}	5.6×10^{4}	5.1×10^{4}	6.6×10^{4}	7.1×10^{4}	6.2×10^{4}	7.1×10^{4}	88±4
	Particulate	5.7×10^{2}	1.8×10^{4}	—	7.5×10^{3}	9.9×10^{3}	5.1×10^{3}	5.4×10^{3}	—	—	1.0×10^{4}	12±4
Hg	Labile	21	3.6×10^{3}	1.7×10^{3}	2.2×10^{3}	2.2×10^{3}	1.4×10^{3}	1.4×10^{3}	1.6×10^{3}	1.8×10^{3}	2.0×10^{3}	31±8
	Particulate	1	6.9×10^{3}	8.0×10^{3}	4.7×10^{3}	4.3×10^{3}	5.0×10^{3}	4.3×10^{3}	3.7×10^{3}	2.9×10^{3}	2.6×10^{3}	69±8
Bag M2												
Cd	Dissolved	2.9×10^{2}	1.4×10^{4}	1.6×10^{4}	1.5×10^{4}	1.2×10^{4}	1.3×10^{4}	1.2×10^{4}	1.3×10^{4}	1.3×10^{4}	1.4×10^{4}	98±1
	Particulate	1.4×10^{4}	1.1×10^{2}	1.0×10^{2}	1.2×10^{2}	61	2.7×10^{2}	4.6×10^{2}	—	1.3×10^{2}	1.4×10^{2}	2±1
Cu	Dissolved	3.5×10^{3}	4.0×10^{4}	3.2×10^{4}	3.2×10^{4}	2.3×10^{4}	2.1×10^{4}	4.1×10^{4}	3.4×10^{4}	3.4×10^{4}	3.7×10^{4}	90±7
	Particulate	5.9×10^{2}	6.7×10^{3}	7.4×10^{3}	7.0×10^{3}	1.3×10^{3}	—	2.7×10^{3}	—	1.0×10^{3}	1.7×10^{3}	10±7
Hg	Labile	22	8.2×10^{2}	4.8×10^{2}	6.4×10^{2}	5.6×10^{2}	5.3×10^{2}	5.7×10^{2}	4.5×10^{2}	3.5×10^{2}	3.7×10^{2}	32±6
	Particulate	6	1.7×10^{3}	1.9×10^{3}	1.6×10^{3}	1.1×10^{3}	1.3×10^{3}	8.4×10^{2}	7.6×10^{2}	6.5×10^{2}	5.6×10^{2}	66±7
	OM[b]	9	15	2	20	28	32	53	49	10	64	2±2

(continued)

Table 2 concluded.

Metal	Phase	April							May			%
		18[a]	18	19	21	23	26	29	2	5	8	
Bag M1												
Cd	Dissolved	3.5×10^2	1.1×10^4	1.2×10^4	8.9×10^4	8.1×10^3	8.4×10^3	8.2×10^3	8.8×10^3	9.3×10^3	9.1×10^3	98±2
	Particulate	—	74	59	68	78	2.0×10^2	3.9×10^2	—	3.2×10^2	1.9×10^2	2±2
Cu	Dissolved	3.7×10^3	3.3×10^4	2.8×10^4	2.3×10^4	1.5×10^4	1.4×10^4	2.8×10^4	2.6×10^4	2.5×10^4	2.5×10^4	92±5
	Particulate	—	2.2×10^3	4.8×10^3	—	1.6×10^3	2.1×10^3	2.1×10^3	—	8.6×10^2	1.0×10^3	8±5
Hg	Labile	41	6.6×10^2	4.7×10^3	3.3×10^2	2.5×10^2	2.2×10^2	2.0×10^2	2.0×10^2	2.2×10^2	2.2×10^2	28±5
	Particulate	0	1.2×10^3	1.3×10^3	8.0×10^2	7.3×10^2	6.0×10^2	6.9×10^2	4.6×10^2	5.2×10^2	3.2×10^2	68±6
	OM[b]	12	6	0	0	39	47	43	40	30	51	4±3
Bag C2, C2												
Cd	Dissolved	3.3×10^2	3.3×10^2	3.8×10^2	3.7×10^2	3.6×10^2	3.4×10^2	2.8×10^2	3.3×10^2	3.6×10^2	3.8×10^2	86±8
	Particulate	1.1×10^2	1.1×10^2	97	90	20	3.2	37	—	—	—	14±8
Cu	Dissolved	3.8×10^3	3.8×10^3	4.0×10^3	3.8×10^3	3.4×10^3	3.8×10^3	4.6×10^3	4.5×10^3	4.4×10^3	4.6×10^3	93±5
	Particulate	5.1×10^2	5.1×10^2	5.1×10^2	5.8×10^2	1.8×10^2	59	2.5×10^2	—	56	1.3×10^2	7±5
Hg	Labile	5	5	10	8	6	7	12	7	12	9	31±17
	Particulate	18	18	6	16	11	10	4	2	2	2	24±16
	OM[b]	24	24	12	8	8	24	10	18	4	22	45±17

[a] Before addition of metals.

[b] OM = organic matter.

correlation with the concentrations of POC and PON because it relates directly to biomass (Fogg 1975). This study supports such a positive correlation.

Figure 8 depicts the relationship between the concentration of chlorophyll *a* in the enclosed ecosystems and the percentage of a given metal in its particulate phase. During the phytoplankton bloom, the relative amount of particulate Zn increased, clearly showing a positive correlation. Although the relative amount of Hg was high in the particulate phase, it showed no correlation with the growth of phytoplankton. Particulate Cd and Cu also showed no correlation with the growth of phytoplankton.

Relationship between heavy metals in the sediment and chlorophyll *a*

The total amount of heavy metal accumulation in the sediment for each sampling day is listed in Table 3. These values are computed from the amount of sediment collected daily in the sediment trap multiplied by the concentration of the given metal in the sediment.

In the case of Cd, Cu, and Hg, their accumulation in the sediment showed no relationship with the growth of phytoplankton or the content of chlorophyll *a*, but

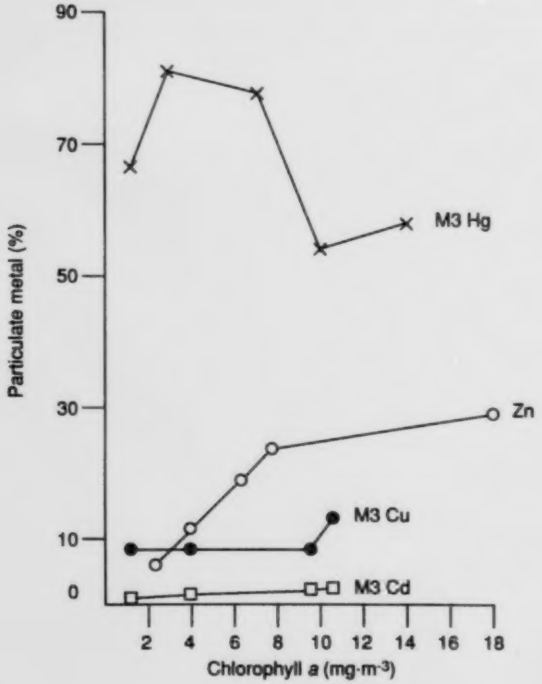

Fig. 8. Relationship between the relative amount of particulate metals [$M_eP/(M_eP + M_eD)$] and chlorophyll *a*. (Note: M_eP, particulate metal; and M_eD, dissolved metal.)

Table 3. Accumulation rate ($\mu g \cdot d^{1-}$) of Cd, Hg, and Cu in the sediment.

Bag	Metal	April					May	
		19	21	23	26	29	2	8
M3	Cd	86	18	15	12	22	—	30
	Cu	2.7×10^3	8.9×10^2	1.3×10^3	7.8×10^2	8.6×10^2	—	7.4×10^2
	Hg	6.8×10^2	4.1×10^2	2.0×10^2	2.4×10^2	3.1×10^2	2.1×10^2	1.4×10^2
M2	Hg	4.6×10^2	2.3×10^2	2.3×10^2	77	77	1.0×10^2	59
M1	Hg	1.6×10^2	1.2×10^2	74	49	30	24	27
S3	Hg	4.1×10^3	1.3×10^2	56	39	20	8.6	10

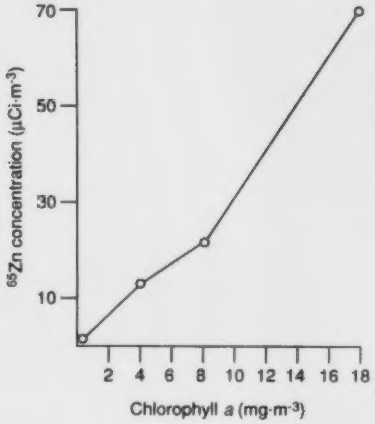

Fig. 9. Relationship between the concentration of ^{65}Zn and chlorophyll *a* in settled material (note: 1 Ci = 37 GBq).

decreased with the amount of settling particles. The close relationship between the concentration of ^{65}Zn in the sediment and the content of chlorophyll *a* (Fig. 9), and hence the growth of phytoplankton, clearly showed that Zn is a bioactive element.

Release of metals and primary productivity in bags treated with sediment

There was some dissolution of Cu and Hg in bag S3 (Fig. 10). Also in the bags treated with sediment, the contents of POC and PON decreased from initial values and never returned to these values over the course of the experiment (Fig. 2). These decreases suggested that primary productivity in the bags treated with sediment significantly suppressed the growth of phytoplankton. Because the slight release of toxic metals in the bags treated with sediment should not have affected primary productivity in the enclosures, it is thought that the adverse response was probably caused by fine sediment particles that reduced light penetration in the water column and, therefore, the growth of phytoplankton. Variations in chlorophyll *a* content and primary productivity in the bags treated with sediment also support this conjecture (Qian et al., this volume).

Conclusions

In marine enclosed ecosystems, the principal source of POC and PON is from the production of phytoplankton because of the greatly reduced input of terrigenous particulates transported by rivers or deposited from the atmosphere. The amounts and characteristics of particulates, and the contents of POC and chlorophyll *a*, etc., become the important parameters in studies investigating the biogeochemical processes of elements.

Biogeochemical processes of heavy metals depend mainly on the nature of the

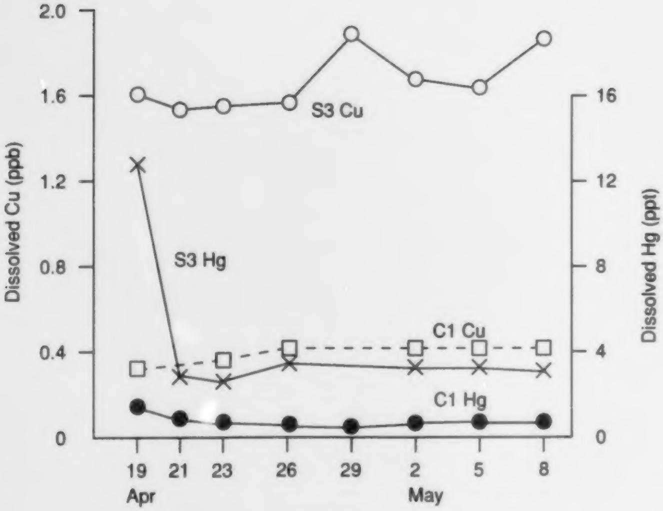

Fig. 10. Dissolved Cu and Hg.

elements. Zinc is bioactive and during a phytoplankton bloom, the amount of particulate Zn increases in proportion with the concentration of chlorophyll *a*. Mercury shows an affinity for particulates. Most of the Hg exists in the particulate phase, but the amount of Hg does not increase proportionately with the occurrence of phytoplankton blooms or the amount of chlorophyll *a*. Cadmium and copper show more affinity for the dissolved phase; therefore, their amounts in the particulate phase do not parallel the abundance of phytoplankton.

Removal rates of various heavy metals in the water column depend on the nature of the element, the abundance of phytoplankton, and the amount of settling particles. In bags treated with metals, Cd, Cu, and Hg constituted 99, 90, and 32% of the total Cd, Cu, and Hg, respectively, with HCl-extractable particulate Cd, Cu, and Hg making up the balance. Concentrations of heavy metals in settled materials revealed that the amount of ^{65}Zn in settled materials increased with the occurrence of phytoplankton, whereas the amounts of Cd, Cu, and Hg did not vary with the abundance of phytoplankton.

There was some dissolution of Cu and Hg in the bags treated with sediment. Suppression of primary productivity observed in these bags was probably caused by fine particles, which attenuate light.

Acknowledgments

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Fogg, G.E. 1975. Primary productivity. *In* Riley, J.P., Skirrow, G., ed., Chemical oceanography: volume 2 (2nd ed.). Academic Press, London, UK. 384–444.

Kuiper, J. 1982. Ecotoxicological experiments with marine plankton communities in plastic bags. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 181–193.

Santschi, P.H. 1982. Application of enclosures to the study of ocean chemistry. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 63–80.

Topping, G., Windom, H.L. 1977. Biological transport of copper at Loch Ewe and Saanich Inlet: Controlled Ecosystem Pollution Experiment. Bulletin of Marine Science, 27, 135–141.

Topping, G., Davies, I.M., Pirie, J.M. 1982. Processes affecting the movement and speciation of mercury in the marine environment. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 167–179.

Wallace, G.T., Seibert, D.L., Holzknecht, S.M. 1982. The biogeochemical fate and toxicity of mercury in controlled experimental ecosystems. Estuarine and Coastal Shelf Science, 15, 151–182.

Release of Heavy Metals from Polluted Sediment in the MEEE Enclosures

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The effect of polluted sediment on the concentration of heavy metals (Cd, Co, Cu, Fe, Ni, Pb, and Zn) in seawater was studied during the 1985 Marine Ecosystem Enclosure Experiment (MEEE) conducted at Xiamen, People's Republic of China. Sediment was added to two 10-m^3 *enclosures with initial concentrations of 11.2 and 112* $mg \cdot L^{-1}$. *About 15 h after treatment, more than 95% of the polluted sediment had sunk to the bottom. At the same time, releases of Cd, Co, Fe, and Pb were at their maximum values. Thereafter, metal concentrations decreased rapidly and returned gradually to their respective background levels. The concentration of dissolved Cu increased rapidly initially to an elevated level and remained at this level to the end of the experiment. There were no significant changes in the concentrations of Ni and Zn before or after treatment. The release of metals depended on their respective natures, the settling rate and size of particles, and biological activity within the enclosures.*

The impact of marine disposal of polluted sediment and dredging materials is a problem currently receiving much attention (Hoff et al. 1982; Li et al. 1986; Wong et al. 1986). There are many important and as yet unresolved questions. Do heavy metals release into the seawater after disposal? What is the release rate and removal time? Are there any effects on the environment? Marine experimental enclosures provide an ideal setup for studying the release of heavy metals from polluted sediment into seawater.

Method and analysis

Polluted sediment was dredged from the bottom at the outfall of the drainage system servicing the Xiamen chemical fertilizer plant.

Before the experiment, experimental bags (nominal volume ca. 10 m^3) were filled with seawater pumped from about 150 m offshore. During the course of the experiment, the salinity of the seawater ranged from 21.0 to 21.5‰; and the pH ranged from 8.1 to 8.47.

Before the experiment started, the seawater in each bag was sampled to establish background concentrations. The polluted sediment was passed through a 125-µm nylon sieve and added, with stirring, to duplicate bags to achieve target concentrations of 11.2 (bag S1) and 112 mg·L^{-1} (bag S3). The two control bags (C1) contained no polluted sediment. All values were the average of the duplicates. The sediments were stirred during addition to the bags. Samples were first taken at 0.5, 2, and 6 h after treatment, and then on days 1, 3, 5, 8, 11, 14, 17, 20, and 27.

Seawater from the upper 3 m was integrally sampled using a peristaltic pump into a large polyethylene bag from which various subsamples were taken. The seawater sample was filtered through a 0.4-µm Nuclepore membrane filter. The filtrate was adjusted with ammonium acetate buffer solution to a pH of 4.5±0.5, complexed with ammonium 1-pyrrolidine dithiocarbamate/diethyl ammonium diethyl dithiocarbamate (APDC/DDDC), extracted with Freon-TF, and then back-extracted with dilute nitric acid. The concentration of heavy metals was determined using a flameless atomic absorption spectrophotometer (Danielsson et al. 1978).

Particles retained on the membrane filter were washed three times with 15 mL of extra-pure water and then dried at 50°C before weighing.

Polluted sediment was soaked in 0.5 *N* HCl, with periodic shaking for 24 h. After centrifugation, the supernatant was used for the determination of HCl-extractable weakly bound metals.

Results and discussion

The polluted sediments sank as soon as they were added to the seawater. Suspended sediment in the integrated 3-m sample accounted for about 48 and 32% of the added sediments in bags S1 and S3 respectively. Six hours after treatment, the suspended sediment in bags S1 and S3 was 17 and 13% respectively. Fifteen hours after treatment, less than 5% of the sediment remained in the top 3 m. Thus, the sinking rate of this polluted sediment in an undisturbed water column was rapid. At the same time, the sediment in particulates carried with it some of the original suspended particulates (ca. 10 mg·L^{-1}) in the seawater. Variations in the concentrations of particulate matter as a function of time are presented in Table 1 and Fig. 1.

Figure 2 illustrates variations in concentrations of dissolved metals (Cd, Co, Cu, Fe, Ni, Pb, and Zn) over time. The release of each heavy metal (Table 2) from the sediment was calculated first as the possible maximum amount assuming the release of all of the weakly bound metal (obtained by extracting the sediment with 0.5 *N* HCl for 24 h). This was compared with the actual measured release, which was the difference between the concentration of the metal in the seawater and its background level. The release of each metal is discussed below.

After the addition of polluted sediment, the concentration of dissolved Cu increased immediately, reaching a maximum 6 h later. On day 3 after treatment, there was a slight decrease in the Cu concentration but it increased again on day 11 and remained at this higher level to day 27. The initial release of Cu was rapid and that Cu did not undergo removal (i.e., absorption or exchange with other ions) during the latter part of the experiment. The increase in Cu concentration on day 11 was probably the result of biological release. In short, Cu exhibits a certain conser-

Table 1. Concentrations ($mg \cdot kg^{-1}$) of suspended particles in seawater.

Time		Bag		
Hours	Days	C1	S1	S3
0		8.15	9.27	9.01
0.5		—	20.58	93.76
2		—	14.66	44.89
6		—	11.20	24.03
	1	8.88	6.55	11.20
	3	8.16	2.68	4.02
	5	0.86	1.17	2.24
	8	0.56	0.52	1.13
	11	1.56	0.50	0.32
	17	0.82	0.44	0.57
	20	1.39	0.79	0.48
	27	2.14	0.92	1.02

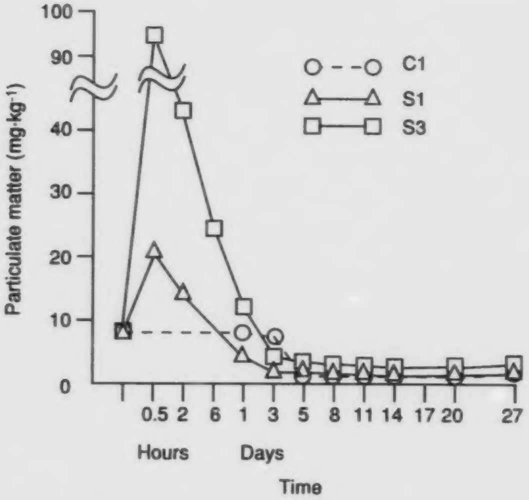

Fig. 1. Concentrations of particulate matter in seawater.

vative behaviour and after being released in seawater, it cannot be removed easily in a short time by natural means.

When polluted sediment was added to bag S3, the amount of Zn in the seawater reached a maximum 2 h later. Zinc remained at this concentration, about double the concentration in the control (C1), over the course of the experiment. Like Cu, the Zn released in the seawater was not adsorbed or scavenged; therefore, it was not removed naturally in a short time. In bag S1, to which with a low amount of sediment was added, the amount of Zn released was very small, and concentrations were similar to those observed in the control.

In bag S3, the concentration of dissolved Cd rose immediately after the addition

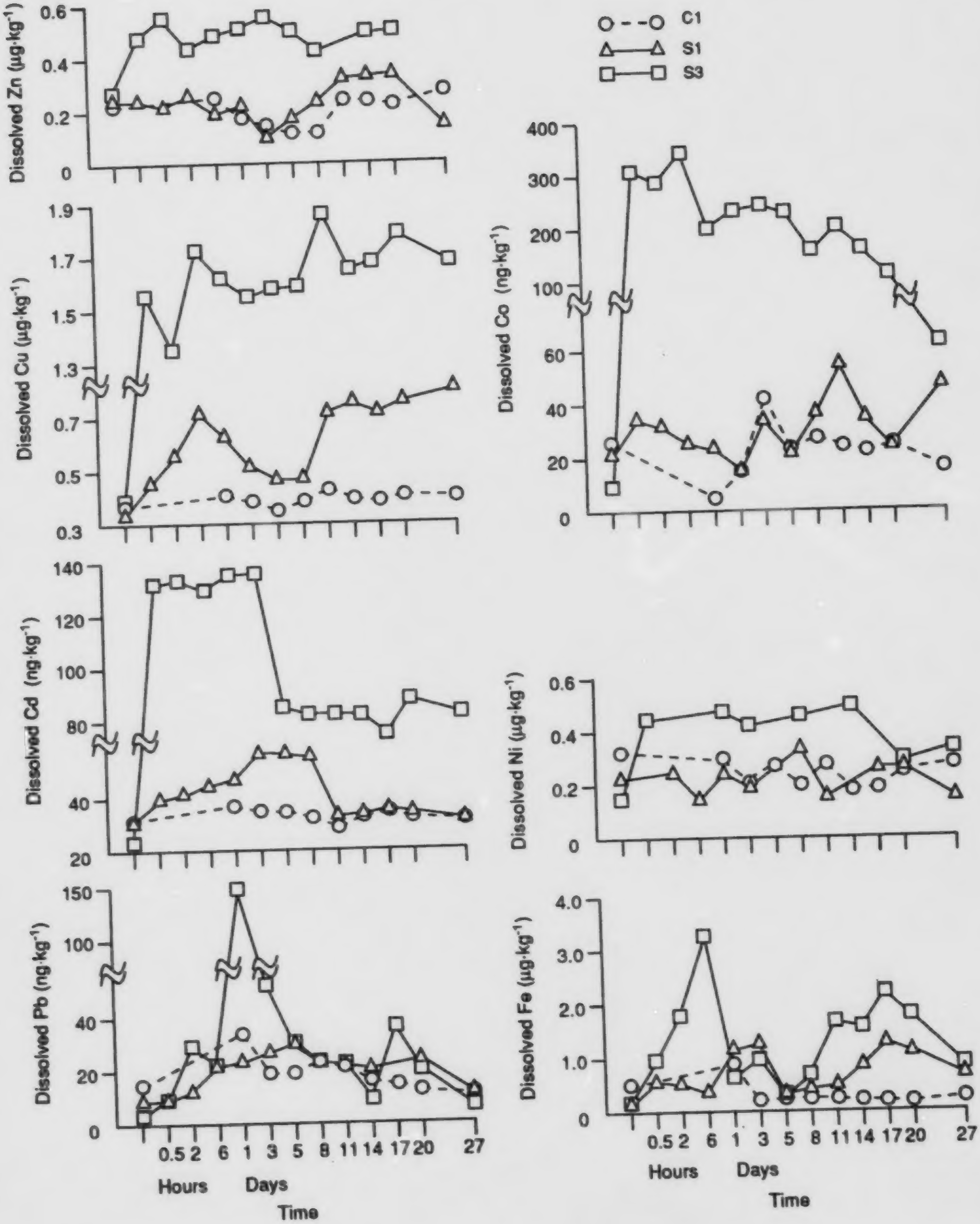

Fig. 2. Variations in the concentrations of dissolved heavy metals in seawater.

Table 2. Release of heavy metals from the polluted sediment in seawater.

	Weakly bound metal ($\mu g \cdot kg^{-1}$)		Actual release ($\mu g \cdot kg^{-1}$)		Release (%)	
	S1	S3	S1	S3	S1	S3
Cu	1.5	15	0.34	1.43	22.7	9.5
Pb	6.6	66	0.0	0.12	0	0.2
Zn	0.33	3.3	0.0	0.36	0	10.9
Cd	0.007	0.07	0.007	0.10	100	100
Co	0.06	0.6	0.036	0.32	60	52.5
Ni	0.02	0.2	0.0	0.2	0	100
Fe	5.36	—	0.98	2.98	0.2	0.06

Note: Concentrations in bags S1 and S3 were 11.2 and 112 $mg \cdot kg^{-1}$ respectively.

of polluted sediment, reaching its maximum 0.5 h later. After 3 d, the Cd concentration decreased rapidly, to about twice its background concentration, and stayed at this level for the remainder of the experiment. In bag S1, the concentration of Cd increased only slightly after treatment. Weakly bound Cd in the sediment had a rather low concentration and all of it would have to be released immediately to account for the dissolved concentration (Table 2). The release and removal of Cd were both fairly rapid. Removal probably resulted from particulate adsorption or ion exchange and need not have involved any biological activity.

The concentration of dissolved Pb in bag S3 reached its maximum 15 h after the addition of polluted sediment and then decreased rapidly to its background level 5 d later. In bag S1, there was no significant change in concentration because the amount of Pb released was very small. This indicates that the release of Pb in seawater is rapid, but that it is also removed quickly, probably by adsorption onto sinking particles.

In the case of Co, the concentration reached its maximum value 6 h after treatment and then decreased slowly. At the end of the experiment, about 80% of the released Co was removed. The concentration of Co in bag S1 did not vary significantly, indicating a rather low release. Removal of Co was probably a result of adsorption onto particles, which then settled to the bottom of the bags.

After the addition of polluted sediment, the concentration of Ni in bag S3 increased to twice its background level; whereas in bag S1, no significant change was observed. Because the amount of weakly bound Ni was relatively low (Table 2), the increase in bag S3 would represent 100% release. In bag S1, even with complete release, the concentration of Ni would only increase by about 10%, which would probably not be discernible here.

The concentration of dissolved Fe in bag S3 reached its maximum 6 h after treatment, but decreased rapidly 1 d later. These observations indicate that Fe is released rapidly and is also easily and completely removed. Removal was probably achieved through precipitation of colloidal Fe compounds, which form when Fe reacts with a strong electrolyte solution, such as seawater. Eleven days after treatment, there was another small peak, which might have resulted from the release of Fe from organisms. In bag S1, as a result of low release and fast removal, the variation in Fe concentration was not as significant as in bag S3.

Conclusions

The polluted sediment sank rapidly in the undisturbed water column, with more than 95% of it sinking to the bottom within 15 h of being added to the seawater. The settling sediment particles also carried with them part of the original suspended particulates in the seawater.

Copper and zinc released to the seawater were not removed naturally in a short period, retaining their postrelease concentrations for more than 20 d. Iron and cobalt released rapidly in the seawater, but Fe was also removed quickly, whereas Co was removed slowly. There was a second release of Fe 11 d after treatment, perhaps from organisms. No such phenomenon was observed for Co.

The release of Pb and Cd reached maximal amounts 15 h after treatment. Thereafter, Pb and Cd were removed quickly, with the concentration of Pb decreasing to near that of the control and the concentration of Cd remaining higher than that in the control.

There was little release of Ni and no noticeable change in the Ni content of the seawater; this is supported by low measured amounts of weakly bound Ni.

Danielsson, L.G., Magnusson, B., Westerlund, S. 1978. An improved metal extraction procedure for the determination of trace metals in seawater by atomic absorption spectrometry with electrothermal atomization. Analytica Chimica Acta, 98, 47–57.

Hoff, J., Thompson, J.A.J., Wong, C.S. 1982. Metal release from mine tailings into seawater — a laboratory study. Marine Pollution Bulletin, 13, 283–286.

Li, J., Johnson, W.K., Wong, C.S. 1986. Laboratory study of heavy metals released from sediment to seawater. Acta Oceanologica Sinica, 8, 314–318 [In Chinese.]

Wong, C.S., Whitney, F.A., Johnson, W.K., Lu, X., Wu, J., Wu, S., Li, J. 1986. The effect of mine tailings on levels of heavy metals in sea water. Acta Oceanologica Sinica, 5, 401–415.

^{65}Zn in the Xiamen MEEE

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In 1985, one of the Xiamen Marine Ecosystem Enclosure Experiment (MEEE) bags was used to study the pathways and fate of ^{65}Zn in the ecosystem by measuring the content of ^{65}Zn in unfiltered seawater, 0.4-µm filtered seawater, suspended particles, and settled particles collected in sediment traps and on the wall of the bag. Chlorophyll a content, and the abundance of phytoplankton and zooplankton were also observed. Results showed that the concentration of ^{65}Zn in seawater decreased exponentially with time, the removal-rate constant (λ_w) was minor –0.0525, and the half-removal time (T_w) was 13.2. During the first 8 d of the experiment, 93–97% of the ^{65}Zn was in the enclosed water body, 3–7% was the suspended particles, and very small amounts were found in settled materials and on the inner wall of the experimental bag. Thereafter, the content of ^{65}Zn in the seawater decreased significantly whereas that in particulates increased to 12–52% of the total ^{65}Zn concentration. Adsorption of ^{65}Zn on settled particles and on the inner wall of the bag also increased slightly. Moreover, the increase in ^{65}Zn on suspended particles corresponded with increases in chlorophyll a content and the number of phytoplankton. These observations indicate that the transfer of ^{65}Zn depends very much on the activity of organisms.

One of the major topics of pollution studies concerns the pathways and removal rates of chemical substances in aquatic systems. An ideal technique for studying these processes is to use radioactive tracers. However, under many circumstances, the addition of radioisotopes to natural ecosystems is not advisable. Thus, an enclosed experimental ecosystem may be a suitable system for applying the radiotracer technique. A thorough review of the use of radioactive tracers in enclosed ecosystems is given by Amdurer et al. (1982).

During the 1985 marine ecosystem enclosure experiment (MEEE) conducted at Xiamen, one of the experimental bags used ^{65}Zn as a tracer. The main pathways and fate of ^{65}Zn in the ecosystem were studied by monitoring the concentrations of ^{65}Zn in unfiltered seawater, 0.4-µm filtered seawater, suspended particles, and settled particles collected in sediment traps and on the wall of the bag.

Methods

Additions of 3.7×10^6 Bq of ^{65}Zn and 175 mg of stable Zn were made to the experimental bag (2 m in diameter × 4 m deep). The initial concentrations of ^{65}Zn

Table 1. Amounts of ^{65}Zn in the seawater and on the wall of the experimental bag at different sampling times.

Sampling time		^{65}Zn concentration		
Day	Time	Unfiltered seawater ($\times 10^5$ Bq·m^{-3})	Filtered seawater ($\times 10^5$ Bq·m^{-3})	Bag wall ($\times 10^2$ Bq·m^{-2})
18 Apr	1700 (0 h)	2.47±0.23	2.29±0.20	—
	1800 (1 h)	2.40±0.23	2.57±0.19	3.76±0.90
	1900 (2 h)	2.71±0.31	2.35±0.26	4.00±0.82
	2100 (4 h)	2.50±0.33	2.66±0.27	4.83±1.21
	2300 (6 h)	2.82±0.27	2.31±0.21	3.92±1.41
19 Apr	0500 (12 h)	2.50±0.34	2.33±0.31	1.88±1.80
	0900 (16 h)	2.76±0.39	2.23±0.24	4.67±1.06
	1700 (24 h)	2.14±0.29	2.19±0.29	2.53±1.06
20 Apr	0900 (2 d)	2.14±0.29	1.63±0.27	2.76±0.74
21 Apr	0900 (3 d)	2.00±0.20	2.08±0.29	3.53±1.06
23 Apr	0900 (5 d)	2.19±0.24	1.92±0.22	3.32±1.42
26 Apr	0900 (8 d)	2.04±0.29	1.83±0.28	7.47±1.42
29 Apr	0900 (11 d)	1.77±0.31	1.64±0.26	12.08±1.64
2 May	0900 (14 d)	1.98±0.28	1.49±0.28	6.02±1.44
5 May	0900 (17 d)	1.57±0.35	1.31±0.28	11.13±1.95
8 May	0900 (20 d)	2.15±0.32	1.10±0.27	34.06±2.38
11 May	0900 (23 d)	1.39±0.22	0.89±0.19	85.40±4.18
15 May	0900 (27 d)	1.78±0.22	1.16±0.24	88.50±6.64

and Zn in the seawater were 3.7×10^2 Bq·L^{-1} and 17.5 µg·L^{-1} respectively. Inside the bag, several sediment traps, each with a cross-sectional area of 72.6 cm^2, were hung at 1- and 3-m depths. Strips of bag material, each with an area of 16.3 cm^2, were also hung at 2-m depth.

On specific days during the experiment, 2 L of seawater from the upper 3 m were sampled integrally from the water column. The amount of ^{65}Zn in unfiltered seawater, seawater filtered with a 0.4-µm Nuclepore filter, filtered suspended particles, and settled material collected in sediment traps and on bag material was determined using a gamma-ray counter fitted with a Ge(Li) probe and an S-80 multichannel analyzer. The dried weights of suspended particles and settled materials were measured using a microbalance. The amount of chlorophyll *a* in the water sample was also determined as well as the abundance of phytoplankton.

Results and discussion

Transfer of ^{65}Zn from the water column

The concentration of ^{65}Zn in the water column decreased exponentially with time (Table 1, Fig. 1) and was similar to results obtained by Santschi et al. (1980) at Rhode Island. It could be assumed that the transfer mechanism of ^{65}Zn from seawater to an other medium followed first-order kinetics. Thus, changes in the number of ^{65}Zn atoms in the water column can be represented by

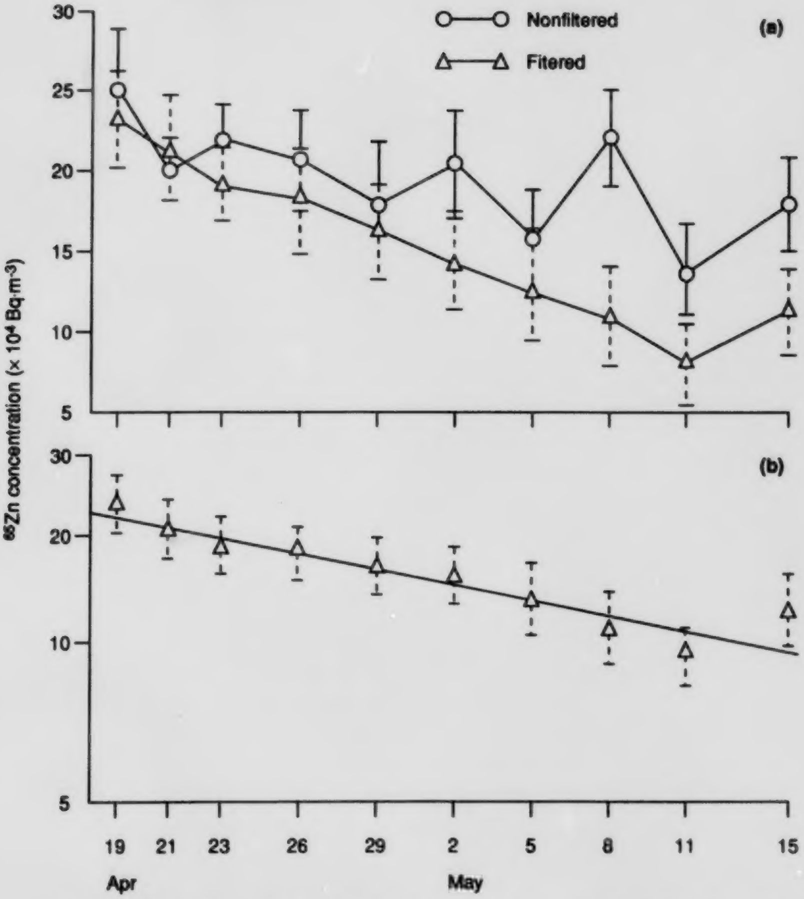

Fig. 1. **(a)** ^{65}Zn radioactivity in nonfiltered and filtered seawater. **(b)** Decay-corrected activities of ^{65}Zn in the water column. (Vertical bars show 1 standard deviation.)

[1] $dN_w/dt = -\lambda_w N_w - \lambda_d N_w$

or its integral form,

[2] $N_w = N^0_w \exp[-(\lambda_w + \lambda_d)t]$

where N^0_w is the number of ^{65}Zn atoms in the water column at time 0; N_w is the number of ^{65}Zn atoms at time t; λ_d is the radioactive decay constant of ^{65}Zn; and λ_w is the transfer-rate constant of ^{65}Zn in the water column.

All radioactivity data were corrected to their values at the start of the experiment. The value of λ_w could be obtained from the slope of the line in Fig. 1. Thus, the half-removal time of ^{65}Zn was estimated by $t_w = \ln 2/\lambda_w$, or 13.2 d.

Concentration of suspended particles and ^{65}Zn radioactivity

Table 2 and Fig. 2 show the concentration of suspended particles and ^{65}Zn radioactivity in the particles at different times. In Table 2, K_D is the partition coefficient of ^{65}Zn between the solid and the liquid phases, i.e.,

Table 2. Concentrations of suspended particles and chlorophyll *a* in the water column, activity of ^{65}Zn in suspended particles, and K_D values.

Sampling time		Concentration of suspended particles (mg·L^{-1})	Zn^{65} in suspended particles (Bq·mg^{-1})	K_D (× 10^4 mL·g^{-1})	Chlorophyll *a* (mg·m^{-3})
Day	Time				
18 Apr	1700 (0 h)	15.40	1.82	0.80	2.83
	1800 (1 h)	12.10	1.59	0.62	—
	1900 (2 h)	9.83	1.83	0.78	—
	2100 (4 h)	9.27	1.93	0.73	—
	2300 (6 h)	8.74	1.78	0.77	—
19 Apr	0500 (12 h)	8.00	1.92	0.82	—
	0900 (16 h)	5.75	2.19	0.98	2.76
	1700 (24 h)	5.47	2.32	1.06	—
20 Apr	0900 (2 d)	4.31	2.40	1.47	—
21 Apr	0900 (3 d)	3.45	1.99	0.96	1.22
23 Apr	0900 (5 d)	2.18	3.17	1.65	1.63
26 Apr	0900 (8 d)	3.81	3.02	1.65	2.17
29 Apr	0900 (11 d)	4.34	5.50	3.35	3.86
2 May	0900 (14 d)	4.77	7.14	4.79	6.30
5 May	0900 (17 d)	4.65	10.1	7.75	7.55
8 May	0900 (20 d)	7.98	16.4	14.9	17.7
11 May	0900 (23 d)	5.46	12.3	13.8	65.8
15 May	0900 (27 d)	4.27	8.40	7.24	—

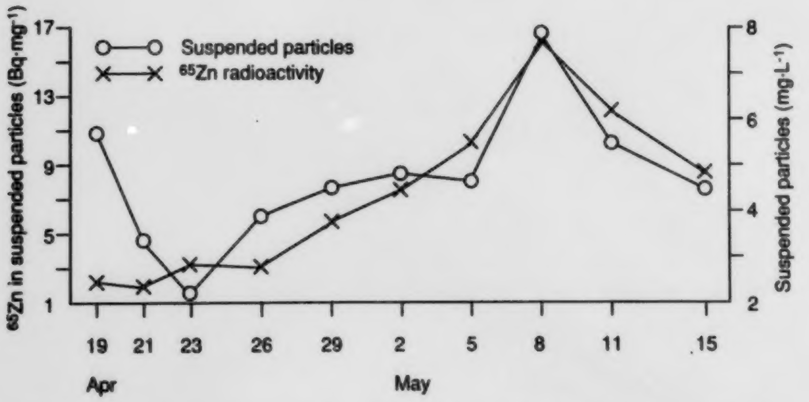

Fig. 2. Concentration of suspended particles and ^{65}Zn radioactivity associated with the particulate phase in the upper 3 m of the water column.

Table 3. Numbers of phytoplankton (individuals·mL^{-1}).

	April						May			
	18	19	21	23	26	29	2	5	8	11
Microflagellates										
1–5 µm	19 467	9 707	4 977	6 560	4 924	9 109	23 618	2 620	65 384	12 310
6–10 µm	2 005	1 337	985	1354	1 723	1 847	739	756	1 900	1 952
11–20 µm	193	756	14	158	123	123	229	176	1 900	1 952
Gymnodinium sp.	53	0	18	70	35	141	—	—	—	—
Cryptophyta	352	281	246	229	211	281	106	70	—	—
Chlorophyta	18	—	—	—	—	—	—	—	—	—
Chrysophyta	—	—	18	35	—	35	—	—	36	87
Euglenophyta	106	18	53	—	—	—	—	—	—	—
Exuviaella sp.	—	—	53	106	264	141	—	—	—	—
Peridinium sp.	—	—	—	53	—	—	—	—	—	—
Silicoflagellineae	—	—	—	—	—	—	18	70	176	—
Total flagellates	22 194	12 117	6 737	8 565	7 280	11 677	24 710	3 692	67 988	14 947
Diatoms	—	—	—	—	—	—	—	—	—	—
Pennate	18	—	—	—	—	18	—	53	—	—
Nitzschia sp.	—	—	35	—	—	—	53[a]	70	—	—
N. closterium	—	18	35	53	—	—	88	123	386	211
N. lorenziana	—	—	—	—	—	—	—	18	—	—
Fragilaria sp.	—	—	—	—	—	—	—	141	1 422	141
Phaedactylum tricornutum	—	—	—	—	—	—	—	18	—	—
Skeletonma costatum	—	—	—	—	—	—	—	35	246	—
Navicula spp	—	—	—	—	—	—	—	458	53	—
Amphiprora sp.	—	—	—	—	—	—	—	36	—	—
Total diatoms	18	18	70	53	—	18	141	370	2 638	405
Others	36	—	—	70+18	—	18	18+15	18	345[b]	580

[a] Two species of Nitzschia included.

[b] Includes 36 ciliates.

[3] $K_D = (\mathrm{Bq}\,^{65}\mathrm{Zn}\cdot\mathrm{g}^{-1}\ \text{particulate})/(\mathrm{Bq}\,^{65}\mathrm{Zn}\cdot\mathrm{mL}^{-1}\ \text{water})$

During the first 5 d of the experiment, the concentration of suspended particles decreased rapidly. On day 1, it decreased from 15.4 $\mathrm{mg\cdot L^{-1}}$ to 5.47 $\mathrm{mg\cdot L^{-1}}$, and on day 5 to 2.18 $\mathrm{mg\cdot L^{-1}}$. At the beginning of the experiment, right after the addition of ^{65}Zn, the water in the bag was stirred thoroughly, which resulted in total suspension of all particulates. The large nonbiotic particles began to sink rapidly to the bottom, thus reducing the amount of suspended particles in the water column. Because the suspended particles had a large surface area, which could adsorb ^{65}Zn from the water, ^{65}Zn radioactivity in these particles also increased with time.

Five days after treatment, there was increasing growth of phytoplankton and the concentration of suspended particles increased gradually to a peak on day 20 (Fig. 3). The ^{65}Zn radioactivity also increased gradually because Zn is a bioessential element that is taken up by organisms. After the peak, the concentration of suspended particles and ^{65}Zn radioactivity decreased because of the death and decay of plankton. Similar trends were observed for chlorophyll *a* and phytoplankton abundance (Tables 2 and 3, Fig. 3).

Sediment flux and ^{65}Zn radioactivity of the settled materials

Variabilities of sediment fluxes and ^{65}Zn radioactivities of settled materials from the upper (1-m) and lower (3-m) sediment traps were similar (Fig. 4). Most of these materials settled to the bottom within the first 24 h. In the lower trap, the flux 2 h after treatment was 10 $\mathrm{mg\cdot cm^{-2}\cdot d^{-1}}$, decreasing to 1.9 $\mathrm{mg\cdot cm^{-2}\cdot d^{-1}}$ 16 h after treatment and to 0.81 $\mathrm{mg\cdot cm^{-2}\cdot d^{-1}}$ 40 h after treatment. For the next 20 d, the flux remained at 0.1–0.3 $\mathrm{mg\cdot cm^{-2}\cdot d^{-1}}$. The ^{65}Zn radioactivity in settled materials increased rapidly from 0.0229 to 0.32 $\mathrm{Bq\cdot mg^{-1}}$ during the first 8 d of the experiment. The increase was a result of particulate adsorption of ^{65}Zn. During the next 8 d, ^{65}Zn radioactivity decreased with time, probably a result of desorption and decay of the particles. Thereafter, ^{65}Zn radioactivity increased significantly again, probably reflecting the high adsorption of plankton.

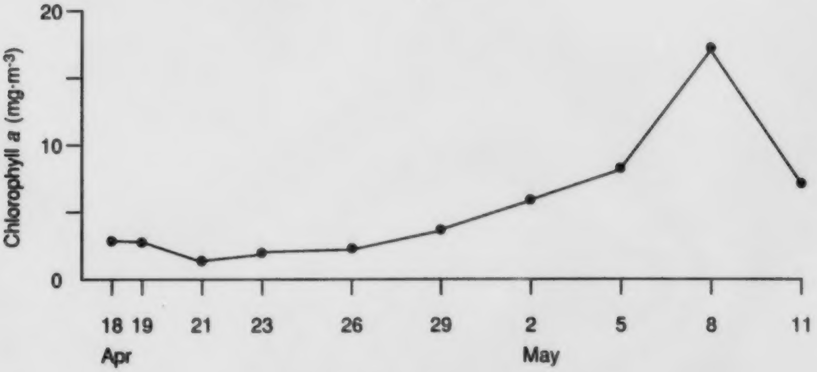

Fig. 3. Chlorophyll *a* in the seawater.

Table 4. Distribution of ^{65}Zn among seawater, in suspended particles, and on the bag wall.

Sampling time		Filtered seawater (%)	Suspended particles (%)	Settled particles (%)	Wall material (%)
Day	Time				
18 Apr	1700 (0 h)	89.1	10.9	0.0	0.0
	1800 (1 h)	92.8	6.9	0.0	0.3
	1900 (2 h)	92.6	7.1	0.0	0.3
	2100 (4 h)	93.4	6.3	—	0.3
	2300 (6 h)	93.3	6.3	0.1	0.3
19 Apr	0500 (12 h)	93.6	6.2	0.1	0.1
	0900 (16 h)	94.2	5.3	0.1	0.4
	1700 (24 h)	94.2	5.5	0.1	0.2
20 Apr	0900 (2 d)	93.5	5.7	0.2	0.3
21 Apr	0900 (3 d)	96.1	3.2	0.3	0.4
23 Apr	0900 (5 d)	95.8	3.4	0.4	0.4
26 Apr	0900 (8 d)	92.7	5.8	0.7	0.8
29 Apr	0900 (11 d)	85.4	12.4	0.9	1.3
2 May	0900 (14 d)	80.0	18.3	1.1	0.7
5 May	0900 (17 d)	71.6	25.8	1.3	1.3
8 May	0900 (20 d)	43.9	52.1	1.1	2.8
11 May	0900 (23 d)	50.3	37.7	1.8	10.1

Adsorption of ^{65}Zn on the wall of the experimental bag

1

There was very little adsorption of ^{65}Zn on the wall of the bag during the first 8 d of the experiment. The amount adsorbed was only $2–5 \times 10^2$ Bq·m^{-2}, or about 0.1–0.4% of the total ^{65}Zn in the system. During the next 8 d, there was a slight increase in adsorption on the wall of the bag, amounting to $6–12 \times 10^2$ Bq·m^2, or 0.7–1.3% of the total ^{65}Zn. Thereafter, adsorption increased rapidly to $11–85 \times 10^2$ Bq·m^{-2}, or 1.3–10% of the total ^{65}Zn.

Partition of ^{65}Zn in various phases

Table 4 lists the partition of ^{65}Zn in seawater, suspended particles and settled materials, and on the wall of the experimental bag at different times during the course of the experiment. During the first 8 d, 93–97% of the ^{65}Zn remained in the water body, 3–7% was in suspended particles, and very little was found in settled materials or on the wall of the bag. After day 8, the amount of ^{65}Zn in the water body decreased significantly to about 44–85% of the total, whereas the amount in the particulates increased to 12–52% of the total. There were also slight increases of ^{65}Zn in settled materials and on the bag wall. Moreover, the increase in suspended particles paralleled the increase in chlorophyll *a* content (Table 2, Fig. 3) and the numbers of phytoplankton (Table 3).

These observations indicate that the transfer of ^{65}Zn depends very much on the amount of suspended particles or on the biological activity. As Zn is a bioessential element, its transfer in an ecosystem is strongly affected by the activity of organisms.

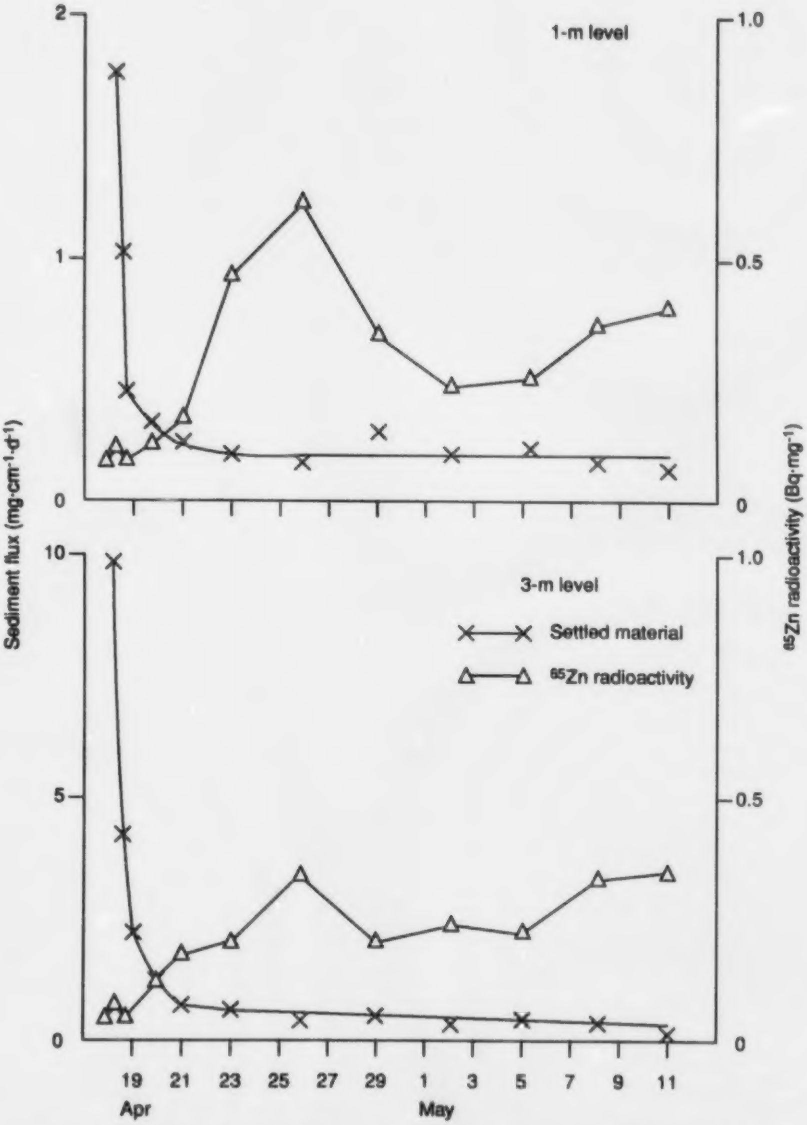

Fig. 4. Fluxes of settled materials collected in sediment traps and ^{65}Zn radioactivity in settled materials at 1- and 3-m depths.

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Amdurer, M., Alder, D.M., Santschi, P.H. 1982. Radiotracers in studies of trace metal behaviour in mesocosms: advantages and limitations. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecocosms. Springer-Verlag, New York, NY, USA. 81–95.

Santschi, P.H., Li, Y-H., Carson, S.R. 1980. The fate of trace metals in Narragansett Bay, Rhode Island: radiotracer experiments in microcosms. Estuarine and Coastal Marine Science, 10, 635–654.

Biogeochemical Processes of Mercury in Marine Enclosures

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This work is part of the enclosed ecosystem study carried out in Xiamen in 1985. Experimental results show that a strong affinity of Hg to particulate organic matter plays an important role in the removal of Hg in enclosed ecosystems. Distribution coefficients of Hg (K_d) in organic matter range from 2 to 15, with direct correlation with particulate organic carbon. Absorption of Hg on particulate organic matter can be described by Freundlich or linear isotherm absorption equations, or both, and the removal rate of Hg can be expressed by first-order equations. First-order removal constants in experimental bags M1, M2, and M3 were 0.050, 0.049, and 0.038; corresponding removal times were, 14, 14, and 18 d respectively.

Since the discovery of Minimata disease in Japan, the problem of Hg pollution in the ocean has received much attention. A wide range of programs have been developed to monitor and study marine Hg pollution. Many studies (Cranton and Buckley 1972; Fitzgerald and Lyons 1973; Wallace 1982; Wallace et al. 1982; Xu et al. 1985, 1986) indicate that the strong complexing of Hg to organic materials significantly affects its speciation, toxicity, and fate.

Experimental ecosystem enclosures have been used to study the biogeochemical processes of Hg (Santschi et al. 1980; Wallace 1982; Wallace et al. 1982). This project, as part of the Xiamen marine ecosystem enclosure experiment (MEEE) jointly sponsored by Canada and China, uses the same approach to study the effect of biogenic organic matter on the biogeochemical processes of Hg.

Methods

Enclosures and additions of Hg

The Hg experiment described in this paper was conducted in a large pool at the Third Institute of Oceanography, Xiamen (MEEE Group, this volume). Five experimental polyethylene bags, each 2 m in diameter × 4.5 m long with a volume of about 10 m^3, were filled with seawater pumped from 150 m offshore during high tide. Nutrient supplements were added to each bag to produce a phosphate concentration of about 0.8 $\mu g \cdot L^{-1}$ in the water column.

Two control bags were designated as C1 and C2. Another three bags M1, M2, and M3 were treated with $HgCl_2$ to produce a total Hg concentration of 185, 258, and 1 060 $ng \cdot L^{-1}$ respectively. After the addition of Hg, the enclosed water was stirred thoroughly and sampling began 2 h later.

Sampling and analysis

Water samples were taken integrally using a peristaltic pump from the upper 3 m of the water column and placed into acid-cleaned polyethylene bags. Subsamples for Hg analyses were stored in acid-washed glass bottles. After each water sample was taken, settled materials were pumped from the bottom of the bags. Both the water and sediment samples were analyzed in the laboratory immediately.

Seawater samples, both unfiltered (for total Hg) and filtered through a 1.2-µm membrane (for dissolved Hg), were digested with a mixture of H_2SO_4 and $K_2S_2O_8$ at room temperature overnight. Mercury analyses were carried out the next day by differential cold vapour atomic absorption (Xu et al. 1983), with a detection limit of 1 $ng \cdot L^{-1}$. The concentration of particulate Hg was the difference between total and dissolved Hg.

The concentration of Hg in sediments, dried at low temperature and digested with a solution of H_2SO_4 and $KMnO_4$ in a 100°C water bath for 2 h, was determined using the same technique as mentioned above, but following the procedure described in a State Oceanic Administration manual (SOA 1979).

Particulate organic carbon was determined using a CHN analyzer (Perkin-Elmer Model 240C). A Turner fluorometer was used to determinate amounts of chlorophyll *a*.

Results and discussion

Temporal variation of chlorophyll *a*

The growth periods of phytoplankton bags M1 and M2 were similar (Fig. 1). During the first 5 d before the phytoplankton bloom, the chlorophyll *a* concentration was low. During the phytoplankton bloom, from days 5 to 11, there was a rapid rise in chlorophyll *a*, then a decline from days 11 to 17. The peak concentration in bag M2 (26.2 $\mu g \cdot L^{-1}$) was more than twice that in bag M1 (11.0 $\mu g \cdot L^{-1}$). In bag M3, the peak of the phytoplankton bloom was delayed 6 d appearing only on day 17. The first 5 d were again the prebloom period; the bloom occurred from days 5 to 17, and then a decline occurred from days 17 to 20. Although this bloom lasted for about 12 d, the peak concentration of chlorophyll *a* was only 15.1 $\mu g \cdot L^{-1}$ (see Qian et al., this volume, for further details).

Temporal variations in particulate organic carbon

Particulate organic carbon (POC) concentration in bags M1, M2, and M3 changed greatly during the experiment (Fig. 2) and followed a trend similar to that

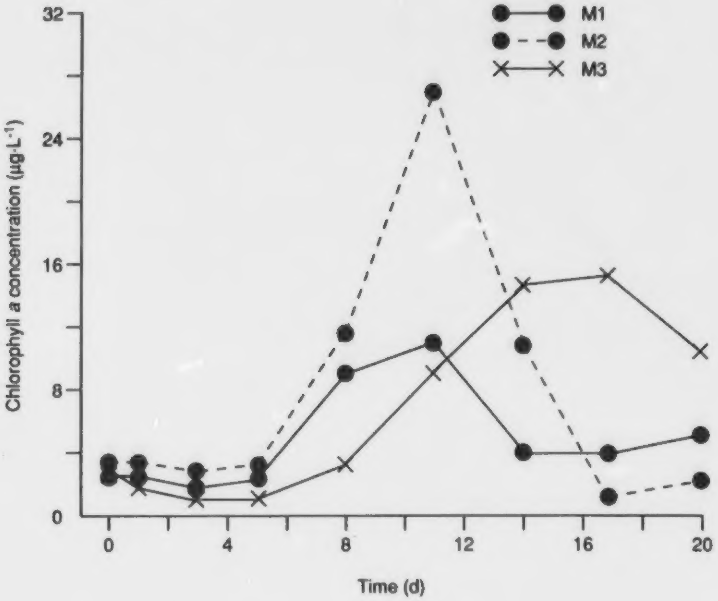

Fig. 1. Variations of chlorophyll *a* with time.

of chlorophyll *a*. Linear regressions of POC against chlorophyll *a* gave correlation coefficients of 0.95, 0.92, and 0.87 for bags M1, M2, and M3 respectively. These results suggest that living phytoplankton carbon accounted for the bulk of POC in the experiment.

Temporal variations in Hg

During the experiment, the total Hg concentration in control bags, C1 and C2, ranged from 1.4 to 4.3 $ng{\cdot}L^{-1}$ (Table 1). Temporal variations in total, particulate, and dissolved Hg concentrations in the three spiked bags are shown in Fig. 3. The total Hg concentration in enclosures M1, M2, and M3 decreased throughout the 20-d experiment by 68, 62, and 57% respectively (Fig. 3).

Mercury in seawater might be removed by three possible pathways: adsorption on the bag wall, evaporative loss, and settling through adsorption on particles. Losses through adsorption on the bag well and evaporation were not determined in this study as these losses were considered to be minor. Adsorption of Hg on sinking particles, on the other hand, was regarded as the most important pathway for Hg removal in the water column (Wallace et al. 1982). This was supported by the results of mass-balance calculations.

Comparing the amounts of Hg added with those amounts in the sediments and in the water, it was found that the amounts of Hg in sediments and in water were 85, 127, and 88%, i.e., a mean of 100 ± 18%, of the known amounts added to bags M1, M2, and M3 respectively. It can be estimated that losses through adsorption on the

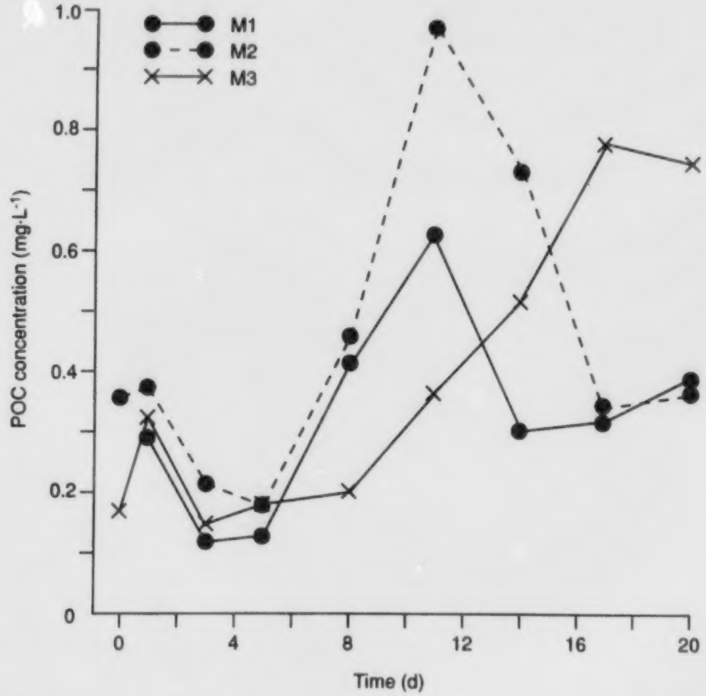

Fig. 2. Temporal variations of total, particulate, and dissolved Hg in (a) bag M1, (b) bag M2, and (c) bag M3.

bag wall and evaporation were less that 18%, although some analytical errors might exist in the results. Based on these results and the strong affinity of Hg to particulate matter, it can also be concluded that the settling of Hg through absorption on particles is the most important pathway for Hg removal from the water column.

Particulate forms of Hg always made up the major fraction of total Hg and the dissolved Hg fraction was minor (Fig. 3). In this experiment, 65% of the total Hg in the water column was readily adsorbed by particles 2 h after the addition of Hg. During the experiment, about 60–80% of the total Hg in the treated bags was in particulate form. The average percentage of 68% was slightly higher that the 53% reported by Wallace et al. (1982), providing evidence for the great affinity of settling particles to Hg.

Table 1. Total mercury concentration in the control bags.

	Day							
Bag	1	3	5	8	11	14	17	20
C1	3.1	2.6	3.3	1.4	1.7	1.8	2.7	2.5
C2	4.0	3.6	2.4	4.3	1.7	1.8	3.0	2.9

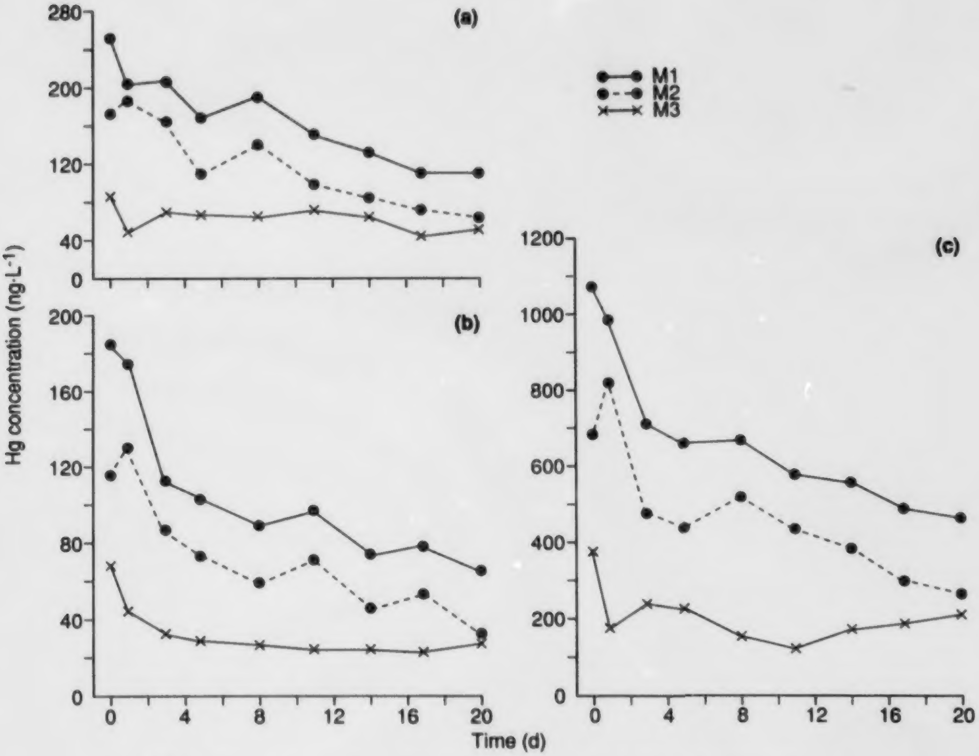

Fig. 3. Temporal variations of particulate (a), dissolved (b), and total (c) Hg in bags M1, M2, and M3.

Distribution coefficients and adsorption isotherms

The partition coefficient of Hg on particles is an important parameter used to gauge the bonding strength of particles with Hg. Olsen et al. (1982) pointed out that organic matter content in particles was a major controlling factor in the adsorption of metals, including Hg on particles. Wallace et al. (1982) used adsorption isotherms to describe the affinity of Hg for POC by normalizing particulate Hg concentrations to POC concentrations and determining the Hg distribution coefficients. The Hg distribution coefficients, K_d, can be represented by the following equation normalized to the concentration of POC:

$$[1] \quad K_d = (\mathrm{Hg})_p \cdot (\mathrm{POC})^{-1} \cdot (\mathrm{Hg})_d^{-1}$$

where $(\mathrm{Hg})_p$ is the concentration of particulate Hg ($\mathrm{ng \cdot L^{-1}}$), POC, is the concentration particulate organic carbon ($\mathrm{mg \cdot L^{-1}}$), and $(\mathrm{Hg})_d$ is the concentration of dissolved Hg ($\mathrm{ng \cdot L^{-1}}$).

For these particles in bags M1, M2, and M3, K_d values ranged between 15 early in the experiment to 2 by the end of the experiment, providing evidence for the great affinity of Hg to organic matter in settling particles. In general, values

Table 2. Distribution coefficients (K_d).

Bag	Day 1	Day 11	Day 20
M1	15	2.2	3.1
M2	7.6	2.8	3.5
M3	13	8.6	1.9

Table 3. Isothermal adsorption equations.

Bag	Type	Equation	*r*	*n*
M1	Linear	$Y = 2.75X - 41$	0.98	8
	Freundlich	$\log Y = 1.10 \log X + 0.14$	0.93	8
M2	Linear	$Y = 2.14X - 0.44$	0.91	9
	Freundlich	$\log Y = 1.20 \log X - 0.14$	0.92	9
M3	Linear	$Y = 1.74X + 496$	0.91	9
	Freundlich	$\log Y = 1.04 \log X + 0.24$	0.91	9

observed before the bloom were higher than those observed during the bloom, and K_d values correlated reciprocally with the concentration of POC (Table 2). Because POC correlates closely with chlorophyll *a*, the decrease in K_d values with increasing POC concentrations might be caused by the "biological dilution effect" of phytoplankton (Ibragim and Patin 1976; Xu et al. 1986).

Assuming that POC was used as the solid adsorbent and dissolved Hg as the solute, and that the adsorption of Hg by POC in the enclosures was in equilibrium under constant temperature (19 ± 2°C), isothermal adsorption equations could be established. Because POC concentrations varied during the experiment, both linear [2] and Freundlich [3] isotherm adsorption equations in the following forms were used to describe the adsorption of Hg by particulate organic matter (POM):

[2] $Y = aX + b$

[3] $\log(Y) = \log(X)/n + k$

in which $Y = (Hg)_p/(POC)$, and $X = (Hg)_d/(POC)$, where $(Hg)_p$, $(Hg)_d$, and POC are as defined for equation [1]. Separate equations for bags M1, M2, and M3 are given in Table 3.

Removal of Hg

Removal of Hg from the water body depends mainly on the adsorption of Hg on the particles and its subsequent sinking. Hence, the removal of Hg can be described by a first-order kinetic equation (Hesslein et al. 1980; Santschi et al. 1980; Wallace 1982; Wallace et al. 1982). The first-order removal rate is obtained using the equation:

[4] $\ln(Hg) = bt + a$

where t is the time (d), (Hg) is the total concentration Hg (ng/L), and b is the total removal rate (d^{-1}).

Table 4. First-order removal rates and half-removal times of mercury.

Bag	Removal rate (b) (d^{-1})	$T_{0.5}$ (d)	*r*	*n*
M1	0.050	14	0.86	9
M2	0.049	14	0.95	9
M3	0.038	14	0.87	9

The half-removal time ($T_{0.5}$) is obtained using the equation:

[5] $T_{0.5} = \ln(2/b)$

Table 4 gives the removal rates and half-removal times of Hg in bags M1, M2, and M3.

Conclusions

The high affinity of Hg to POM was observed and high distribution coefficients for Hg, ranging between 2 and 15, were determined. It was found that the concentration of POC in the water column has an effect on the distribution coefficients of Hg. Both linear and Freundlich adsorption isotherms could be used to describe the adsorption of Hg on POM.

Removal of Hg from the water column was mainly a result of the adsorption of Hg on POM and its subsequent sinking. Thus, the removal of Hg can be described by first-order removal equations.

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Cranton, R.E., Buckley, D.E. 1972. Mercury pathways in a river and estuary. Environmental Science and Technology, 6, 274–278.

Fitzgerald, W.F., Lyons, W.B. 1973. Organic mercury compounds in coastal waters. Nature, 242, 452–453.

Hesslein, R.H., Broecker, W.S., Schindler, D.W. 1980. Fates of metal radiotracers added to a whole lake: sediment–water interactions. Canadian Journal Fisheries and Aquatic Sciences, 37, 378–386.

Ibragim, A.M., Patin, S.A. 1976. Effect of mercury, lead, cadmium and copper on primary production and phytoplankton in some coastal regions of the Mediterranean and Red seas. Oceanology, 15, 589–591.

Olsen, C.R., Cutshall, N.H., Larson, I.L. 1982. Pollutant-particle associations and dynamics in coastal marine environments: a review. Marine Chemistry, 11, 501–533.

Santschi, P.H., Li, Y.H., Carson, S.R. 1980. The fate of trace metals in Narragansett Bay, Rhode Island: radiotracer experiments in microcosms. Estuarine and Coastal Marine Science, 10, 635–654.

SOA (State Oceanic Administration). 1979. The standards for marine pollution surveys. SOA, Xiamur, Peoples Republic of China 155–156. [In Chinese.]

Wallace, G.T., Jr. 1982. The association of copper, mercury and lead with surface-active organic matter in coastal seawater. Marine Chemistry, 11, 379–394.

Wallace, G.T., Jr., Seibert, D.L., Holzknecht, S.M., Thomas, W.H. 1982. The biochemical fate and toxicity of mercury in controlled experiment ecosystems. Estuarine, Coastal and Shelf Science, 15, 151–182.

Xu, K., Wu, L., Wang, R. 1983. Direct determination of trace mercury in seawater by the differential cold vapour atomic absorption method. Acta Oceanologica Sinica, 2, 52–59.

Xu, K., Wu, L., Zhan, X. 1985. Concentration of mercury and its relationship to chlorophyll *a* in the Xiamen Xigang harbour and Jiulong Jiang Estuary. Taiwan Strait, 4, 129–134. [In Chinese.]

Xu, K., Wu, L., Zhen, C., Chen, S., Zhan, X. 1986. The uptake of mercury by phytoplankton in the marine environments. Acta Oceanologica Sinica, 8, 53–65. [In Chinese.]

Part III

China–Canada MEEE Experiments

B. Hydrocarbons

Response of Bacterioplankton to Corexit 9527 and Corexit-Dispersed Shengli Crude Oil: Marine Ecosystem Enclosed Experiments

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The response of bacterioplankton in marine ecosystem enclosures to Corexit 9527 and Corexit-dispersed Shengli crude oil was monitored over a 15-d period in Xiamen Bay, People's Republic of China. Corexit 9527 and Corexit-dispersed crude oil stimulated bacterial productivity. Biodegradation was identified as the major process contributing to rapid elimination of short-chain n-alkanes in Corexit-dispersed crude oil. After 15 d, radiotracer analyses of an n-(1-^{14}C) hexadecane-labeled fraction in the Corexit-dispersed oil showed that more than 25% had been metabolized to CO_2, 3.4% was in the suspended particulate fraction, 12.4% was in sedimentary material, and 1.0% was in the dissolved organic fraction. Significant alterations in species composition of indigenous oil-degrading bacteria within the enclosures were observed after additions of Corexit 9527 and Corexit-dispersed oil.

New generations of "low-toxicity" chemical dispersants are now advocated for cleaning up oil spills. Although the aquatic toxicology of these dispersants and the dispersed oil has been studied extensively (EPS 1982; Allen 1984; Wells 1984), most environmental-impact studies have been laboratory oriented and species specific for higher plants and animals. Little attention has been paid to the effect of oil and dispersants on microorganisms in the marine ecosystem despite their ecological significance in organic carbon production, nutrient regeneration, and microbial degradation of oil (Morita 1977; Peterson 1980; Cretney et al. 1981; Azam et al. 1983).

Recent studies have shown that temperature (Axiak and Schembri 1984) and nutrient conditions (Foght and Westlake 1982) significantly alter the toxicity of chemically dispersed oil. Thus, prevailing environmental conditions in specific regions should be accounted for when designing specific toxicity tests to evaluate the possible biological effect of using dispersants. During a multidisciplinary study employing marine ecosystem enclosures (MEEs) (Menzel and Case 1977; Parsons et al. 1984a), the impact of Corexit 9527 and Corexit 9527-dispersed crude oil (at concentrations expected during actual field applications) on the bacterioplankton of a subtropical marine ecosystem was evaluated.

Methods

Four MEEs (each 2 m in diameter × 6 m deep) were launched simultaneously on 21 May 1986 in Xiamen Bay, China (24° 32′ 30″ N, 118° 11′ 18″ E), by pulling the bags from a depth of 5 m to the surface, thereby capturing about 14 m^3 of seawater in each enclosure. Within 1 h of the launch, a mixture of inorganic nutrients (nitrate:silicate:phosphate = 15:10:0.5 $\mu g \cdot L^{-1}$) was added to the 0- to 3-m layer of the water column using a diffusion assembly similar to that described by Topping and Windom (1977).

On 23 May (day 1), 15 g of Corexit 9527, a concentrate dispersant composed of three nonionic surfactants and one anionic surfactant in a solvent of glycol ethers (Canevari 1971; Wells et al. 1985), distributed by Exxon Chemicals Company, was injected into the upper layer (0–3 m) of MEE-2 (the dispersant enclosure) using the diffuser assembly. A mixture of 15 g of Corexit 9527, 150 g of Shengli crude oil, and 3.7 MBq of n-(1-^{14}C) hexadecane (specific activity: 1.98 $GBq \cdot mmol^{-1}$) was similarly injected into MEE-3. The initial concentrations of dispersant, crude oil, and ^{14}C-labeled hexadecane in the 0–3 m depth zone of MEE-3 immediately after treatment were 1.5 $mg \cdot L^{-1}$, 15 $mg \cdot L^{-1}$, and 370 $Bq \cdot L^{-1}$, respectively. MEE-1 was designated as a "control" enclosure and except for the addition of the radioisotope and MEE-4 was an experimental replicate of MEE-3.

Samples were collected daily from days 1 to 5, and at 2–3 d intervals thereafter until day 15. Integrated seawater samples (0–3 m) were obtained from the enclosures through acid-rinsed polyvinyl chloride (PVC) tubing attached to a peristaltic pump (Little Giant Model LG-300). Samples for radiotracer studies were collected using Niskin bottle casts. A pumping system connected to a sediment collector attached to the conical-shaped bottom of the enclosures (Lee et al. 1978) was used to recover sedimentary material.

Following procedures described by Wong et al. (1984), oil concentrations in the enclosures were measured by direct weighing of extracted oil and changes in oil composition were determined from ion chromatograms. Particulated oil concentrations in the water column were estimated by quantifying the amount of ^{14}C-labeled material retained on filters (0.4-µm Millipore HA) after filtration of 500-mL aliquots. Remaining ^{14}C-labeled oil fractions in the filtrate were recovered by dichloromethane extraction. Carbon-14 CO_2 was recovered from the seawater samples by acidification (1 mL, 18 *M* H_2SO_4) and bubbling (15 min) of 500-mL samples in an enclosed "continuous loop" system containing a CO_2 trap. All radioactivity measurements were obtained by liquid scintillation counting with 10 mL of Aquasol cocktail (NEN).

Primary productivity was measured by monitoring ^{14}C-bicarbonate uptake in

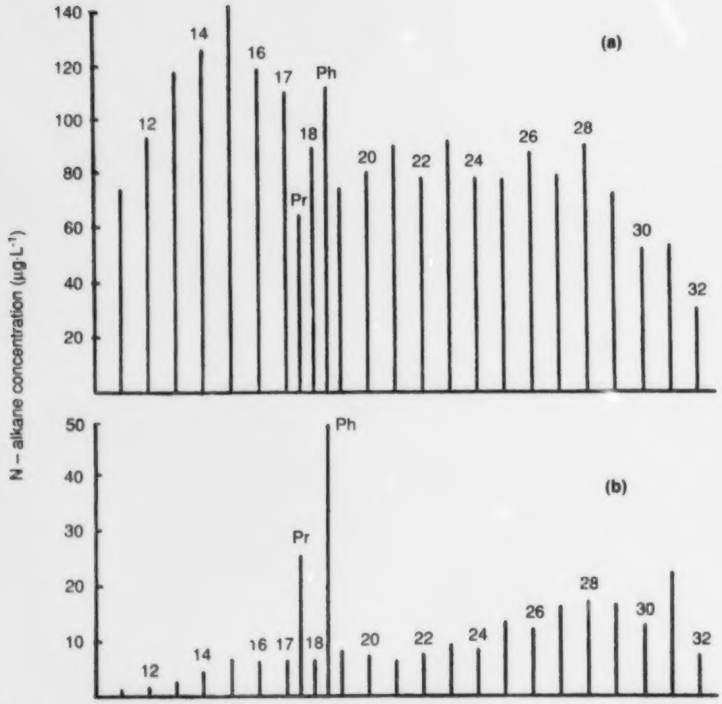

Fig. 1. Relative concentrations of n-alkanes (numbered by chain length) and isoprenoids pristane (Pr) and phytane (Ph) on day 1 (a) and day 5 (b).

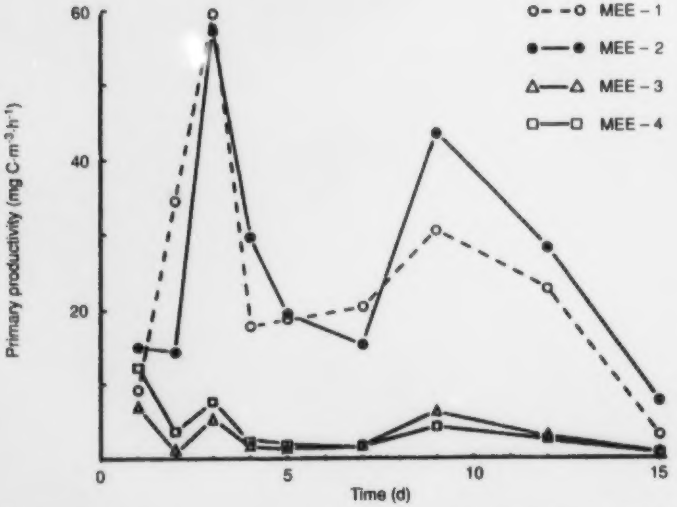

Fig. 2. Changes in primary productivity in the enclosures after oil and dispersant additions on day 1 (all values integrated, 0–3 m).

samples incubated in situ as outlined by Parsons et al. (1984b). Heterotrophic bacterioplankton production was estimated using a method based on incorporating ^{3}H-thymidine into deoxyribonucleic acid (DNA) as described by Fuhrman and Azam (1982).

Bacteria were enumerated by direct microscopic observation of acridine orange-stained samples under epifluorescent illumination (Hobbie et al. 1977). Species of isolated oil-degrading bacteria were identified to the genus level using standard procedures outlined in Bergey's manual (Buchanan and Gibbons 1974). Phytoplankton, ciliates, and microflagellates in water samples preserved in Lugol's solution and concentrated in settling chambers were enumerated using inverted microscopy. Zooplankton samples, collected in 0- to 3-m vertical net tows (202-µm mesh), preserved in 4% formalin, and subsampled with a Folsom splitter, were identified and enumerated with the aid of a dissecting microscope (Parsons et al. 1984b).

Results

Gravimetric analysis showed that between days 1 and 5, dissolved and particulate Corexit-dispersed oil fractions in the water column declined from 3.43 and 18.30 mg·L^{-1} to 0.75 and 4.30 mg·L^{-1} respectively. Shorter carbon chain n-alkanes were preferentially degraded and the ratios of C_{17}/pristane and C_{18}/phytane declined from 1.72 and 0.79 to 0.27 and 0.13, respectively, within 5 d (Fig. 1).

Except for a 1-d inhibition period immediately after the addition of Corexit 9527, primary production values in the control (MEE-1) and the enclosure treated with Corexit 9527 (MEE-2) were remarkably similar throughout the experiment, i.e., increasing from day 1 to a peak on day 3, followed by a rapid decline and

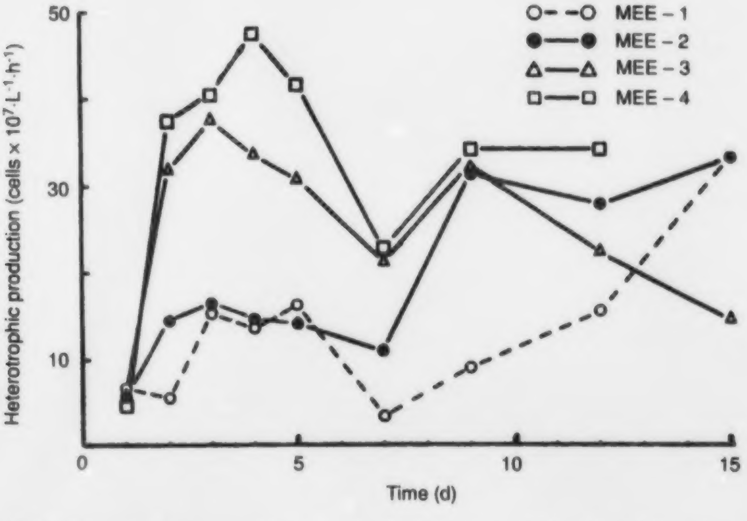

Fig. 3. Changes in heterotrophic production in the enclosures after oil and dispersant additions on day 1 (all values integrated, 0–3 m).

recovery on day 7 to a second peak on day 9 (Fig. 2). In contrast, primary production in the oiled enclosures (MEE-3 and MEE-4) declined immediately after the addition of Corexit-dispersed oil and remained low, with little apparent recovery.

Heterotrophic activity in MEE-3 and MEE-4 increased to a peak on days 3 and 4 immediately after the addition of Corexit-dispersed crude oil. This was followed by an initial decline and later recovery after day 7. Although heterotrophic production in the control (MEE-1) and the enclosure treated with dispersant (MEE-2) increased only slightly during the first 3 d of the experiment, compared with enclosures treated with dispersed oil, a significant increase was observed between days 7 and 15 (Fig. 3). Over the experimental period, total heterotrophic production in the water column of the enclosure treated with Corexit 9527 (MEE-2) was significantly higher than that in the control enclosure (MEE-1).

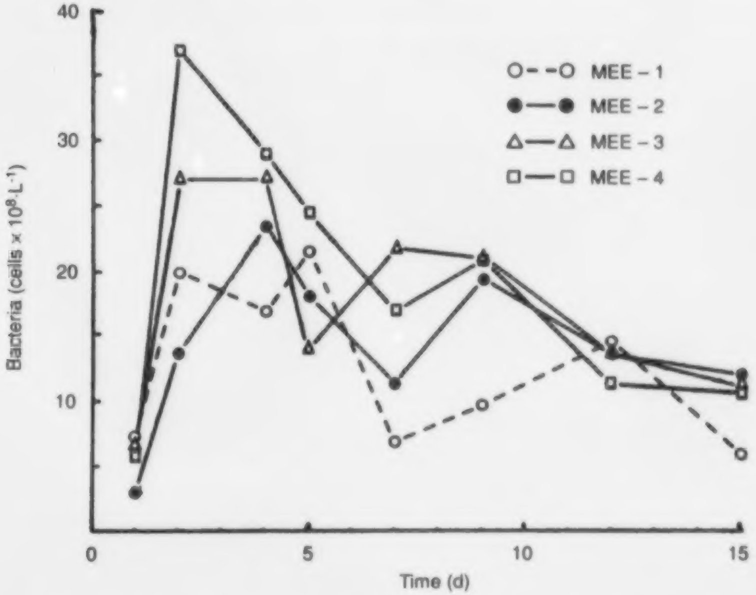

Fig. 4. Changes in bacterial numbers in the enclosures after oil and dispersant additions of day 1 (all values integrated, 0–3 m).

Table 1. Oil-degrading bacteria in the enclosures during the 15-d experimental period.

	MEE-1	MEE-2	MEE-3
Pseudomonas spp	+	+	+
Gluconobacter spp	+	+	+
Acinetobacter spp	+	+	
Corynebacterium spp	+		+
Achromobacterium spp		+	+
Aeromonas spp			+
Vibrio spp			+

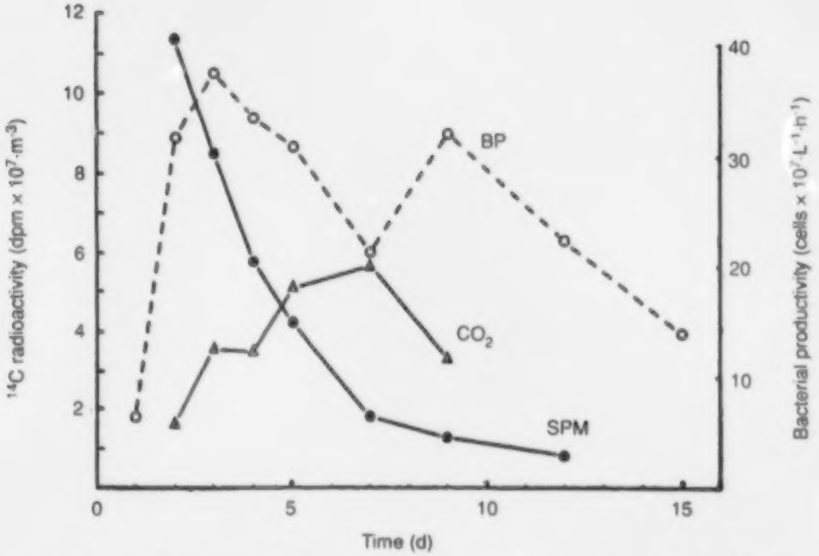

Fig. 5. Integrated (0–6 m) ^{14}C-radiotracer data representing the water column of MEE-3 and illustrating the time-series distribution of suspended particulate matter (SPM), CO_2, and bacterial productivity (BP).

Bacterial numbers in enclosures increased between days 1 and 2. With the exception of day 5, bacterial standing stocks were higher in MEE-3 and MEE-4 than in MEE-1 and MEE-2 (Fig. 4) during the first 9 d of the experiment. The greatest species diversity and numbers of oil-degrading bacteria were observed in MEE-3, which had been treated with Corexit-dispersed crude oil (Table 1). The time-series distribution of ^{14}C-labeled particulate matter and ^{14}C-CO_2 in the water column for MEE-3 (Fig. 5) showed that, during the first 7 d, ^{14}C-hexadecane in the particulate oil was rapidly lost from the water column while ^{14}C-CO_2 increased as a result of respiration. By day 15, 3.4% of the added radiotracer was in the suspended particulate fraction (>0.45 μm), 1.0% was in the form of dissolved organic material, 12.4% was associated with sedimentary material, and >25% was respired as CO_2.

Discussion

To minimize surface evaporation of volatile components and coalescence of dispersed oil at the air–water interface, Corexit 9527 and Corexit-dispersed Shengli crude oil were mixed into the 0–3 m depth zone of the water column by subsurface injection. One day after treatment, the Corexit-dispersed crude oil and the dispersant were homogeneously mixed throughout the water column of the enclosures as a result of convection and diffusion processes. Assuming no losses, final concentrations of oil and dispersant in the enclosures would be 1.0 ppm and 10.0 ppm respectively — concentrations similar to those encountered under actual field applications (Blackall and Sergy 1983; Linton and Koons 1983).

In response to the addition of nutrients and the stabilizing effect of the enclosure walls on the water column, blooms of centric diatoms, along with a few microflagellates and pennate diatoms, were stimulated in MEE-1 within 3 d of launching the enclosure. After day 3, primary production in the water column of MEE-1 and MEE-2 declined rapidly as a result of nutrient depletion and sedimentation processes. In MEE-2, primary productivity was inhibited immediately by the addition of Corexit 9527, suggesting that 1.0 ppm Corexit inhibited phytoplankton growth. However, recovery of primary production processes on day 2 by species selection and adaptation to dilution of the dispersant resulted in an ecological response similar to that observed in the control. In contrast, primary production was severely depressed and did not recover during the experimental period in the enclosures treated with dispersed crude oil (MEE-3 and MEE-4). Many studies have proven that hydrocarbons inhibit phytoplankton photosynthesis (Gordon and Prouse 1973), cell division (Mironov and Lanskaya 1968), and growth (Winters et al. 1976; Hsiao 1978; Fabregas et al. 1984).

Primary productivity results obtained in the subtropical waters of Xiamen Bay correspond with conclusions previously reported for arctic (Hsiao et al. 1978) and temperate (Lee et al. 1985) waters, suggesting that oil mixtures dispersed with Corexit are more toxic than Corexit alone. Furthermore, the toxicity of dispersed oil mixtures to marine algae may be correlated with the efficiency of the dispersant (Bratbak et al. 1982), so that toxic effects would be more pronounced with increasing effectiveness (i.e., greater availability of petroleum hydrocarbon concentrations in the water column).

Enclosure studies by Parsons et al. (1984a) showed that, although Corexit at concentrations as low as 2.0 ppm stimulated heterotrophic production (as measured by the uptake of ^{14}C-glucose), it did not alter the ecological structure of the planktonic food chain. In the present experiment, although values were generally higher in the enclosure amended with Corexit 9527, similar trends in heterotrophic productivity (measured as thymidine incorporation into DNA) and bacterial standing stock were observed in MEE-1 and MEE-2. The immediate stimulation of bacterial productivity observed in MEE-2 after addition of Corexit 9527 may be correlated with an increase in the liberation of organic material from phytoplankton attributed to responses by the phytoplankton to the Corexit (cell lysis or extracellular release) as shown in the primary productivity data.

Contrary to the conclusions of a previous mesocosm study conducted in temperate waters, which showed that oils in seawater at concentrations above 300 $\mu g \cdot L^{-1}$ significantly inhibited heterotrophic uptake and mineralization of ^{14}C-glucose (Hodson et al. 1977), heterotrophic uptake of ^{3}H-thymidine in MEE-3 and MEE-4 increased immediately after the addition of Corexit-dispersed crude oil to values three to four times higher than those observed in MEE-1 and MEE-2. ^{14}C-glucose uptake and respiration has been used extensively used as an index of microbial heterotrophic potential, because it is generally available to and readily used by marine microbial heterotrophs. However, if there is a shift in metabolism or species composition in favour of hydrocarbon oxidizers, it may not be an accurate index of total bacterial activity. Thymidine incorporation into DNA, on the other hand, measures cell growth and, therefore, represents the integration of many metabolic activities.

The increase and decline in heterotrophic productivity and bacterial standing stock within the first 7 d of the experiment in MEE-2, MEE-3, and MEE-4 suggested that indigenous bacteria in Xiamen Bay metabolized Corexit 9527 and Corexit-dispersed crude oil. Furthermore, high numbers of oil-degrading bacteria were observed on Corexit-dispersed oil droplets attached to glass microscope slides suspended in the water column of MEE-3 (Lin et al. this volume) and the decline in pelagic bacterial productivity and standing stock on days 3 and 4 corresponded with the loss of easily degradable n-alkane fractions of the oil (Fig. 1).

Although Parsons et al. (1984a) and Lee et al. (1985) have suggested that Corexit-dispersed crude oil may indirectly alter bacterial biomass by stimulating or depressing the growth of bactivores, such as microflagellates, ciliates, and appendicularians, such an impact by grazing pressure does not appear to be a significant factor in the present study because the variation in microflagellate numbers was small and ciliates and appendicularians were seldom observed in samples (MEEE Group 1986).

The potential biodegradation rate of crude oil in the marine environment is positively correlated to the type and numbers of oil-degrading bacteria. Areas with high background concentrations of oil generally have diverse populations of oil-degrading or oleoclastic bacteria (Ni et al. 1983). Results from the present study showed that the diversity of oil-degrading bacterial species in the water column of MEE-3, treated with Corexit-dispersed crude oil, was higher than that observed in MEE-1 and MEE-2 (Table 1). Whereas four genera of oil-degrading bacteria were isolated in MEE-1 and MEE-2, six genera of gram-negative bacteria with high oil-degradation potential were isolated in MEE-3. It is generally recognized that a single bacterial strain can only degrade a few of the many components found in a typical crude oil. Thus, simultaneous growth of many species would support degradation of more complex components (Zhou et al. 1983) and enhance degradation rates.

In MEE-3, most of the n-alkanes in the Corexit-dispersed crude oil were lost by day 5 (Fig. 1). Field experiments investigating biodegradation of oil by indigenous organisms have generally demonstrated preferential utilization of n-alkanes relative to the isoprenoids pristane and phytane (McKenzie and Hughes 1976; Ward et al. 1980). The decline in the ratio of nC_{17} to pristane and nC_{18} to phytane observed in MEE-3 suggests that biodegradation is a principal process contributing to the loss of low volatility components in the Corexit-dispersed crude oil. Radiotracer studies using n-(1-^{14}C) hexadecane confirmed that the Corexit-dispersed crude oil was rapidly biodegraded. Fifteen days after the addition of the mixture of Corexit 9527, Shengli crude oil, and n-(1-^{14}C) hexadecane to MEE-3, 3.4% of the added radiotracer was recovered in the suspended particulate fraction, 12.4% was in the sediments, 1.0% was in the dissolved organic pool, and 25% respired as $^{14}C\text{-}CO_2$. These values are conservative because only dichloromethane-extractable dissolved organics were recovered and atmospheric losses of $^{14}C\text{-}CO_2$ and volatile ^{14}C-metabolites by evaporation were not measured. The results are similar to those obtained in a Marine Ecosystems Research Laboratory (MERL) mesocosm experiment, conducted during the summer months in Narragansett Bay, RI, USA (Wakeham and Canuel 1986), that demonstrated conclusively that mineralization of aliphatic hydrocarbons plays a major role in their removal from the water column.

Acknowledgments

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Allen, T.E., ed. 1984. Oil spill chemical dispersants: research, experience and recommendations. American Society for Testing and Materials, Philadelphia, PA, USA. Special Technical Publication 840, 465.

Axiak, V., Schembri, J.L. 1984. Toxicity of crude oil and crude oil plus dispersant mixtures on the marine diatom *Phaeodactylum tricornutum*. Journal des Études de Pollution, 7, 763–768.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A., Thingstad, F. 1983. The ecological role of water column microbes in the sea. Marine Ecology Progress Series, 10, 257–263.

Blackall, P.J., Sergy, G.A. 1983. The BIOS project: an update. *In* Proceedings of 1985 Oil Spill Conference 28 February–3 March, San Antonio, TX, USA. American Petroleum Institute, Washington, DC, USA. Publication 4356, 451–455.

Bratbak, G., Heldal, M., Knutsen, G., Lien, T., Norland, S. 1982. Correlation of dispersant effectiveness and toxicity of oil dispersants towards the alga *Chlamydomonas reinhardi*. Marine Pollution Bulletin, 13, 351–353.

Buchanan, R.E., Gibbons, N.E. 1974. Bergey's manual of determinative bacteriology (8th ed.). Williams and Wilkins, Baltimore, MD, USA.

Canevari, G.P. 1971. Oil slick dispersant and method. United States Patent Office, Washington, DC, USA. Patent 3793218 (Filed 1971, Patented 1974), 4 pp.

Cretney, W.J., Macdonald, R.W., Wong, C.S., Green, D.R., Whitehouse, B., Geesey, G.G. 1981. Biodegradation of a chemically dispersed crude oil. *In* Proceedings of the 1981 Oil Spill Conference, 2–5 March 1981, Atlanta, GA, USA. American Petroleum Institute, Washington, DC, USA. Publication 4334, 37–43.

EPS (Environmental Protection Service). 1982. Oil dispersants in Canadian seas — research appraisal and recommendations. *In* Sprague, J.B., Vandermeulen, J.H., Wells, P.G. ed., Economic and Technical Review. Environment Canada, Ottawa, ON, Canada. Report EPS-3-EC-82-2, 185 pp.

Fabregas, J., Herrero, C., Veiga, M. 1984. Effect of oil and dispersant on growth and chlorophyll *a* content of the marine microalga *Tetraselmis suecica*. Applied and Environmental Microbiology, 47, 445–447.

Foght, J.M., Westlake, D.W.S. 1982. Effect of the dispersant Corexit 9527 on the microbial degradation of Prudhoe Bay oil. Canadian Journal of Microbiology, 28, 117–122.

Fuhrman, J.A., Azam, F. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Marine Biology, 6, 109–120.

Gordon, D.C., Prouse, N.J. 1973. The effects of three oils on marine phytoplankton photosynthesis. Marine Biology, 22, 329–333.

Hobbie, J.E., Daley, R.J., Jasper, S. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology, 33, 1 225–1 228.

Hodson, R.E., Azam, F., Lee, R.F. 1977. Effects of four oils on marine bacterial populations: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 423–430.

Hsiao, S.I.C. 1978. Effects of crude oils on the growth of arctic marine phytoplankton. Environmental Pollution, 17, 93–107.

Hsiao, S.I.C., Kittle, D.W., Foy, M.G. 1978. Effects of crude oils and the oil dispersant Corexit on primary production of arctic marine phytoplankton and seaweed. Environmental Pollution, 15, 209–221.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parsons, T.R., Lalli, C.M., Wu, J. 1985. Microbial response to crude oil and Corexit 9527: Seafluxes enclosure study. Microbial Ecology, 11, 337–351.

Lee, R.F., Gardner, W.S., Anderson, J.W., Blaylock, J.W., Barwell-Clarke, J. 1978. Fate of polycyclic aromatic hydrocarbons in controlled ecosystem enclosures. Environmental Science and Technology, 12, 832–838.

Linton, T.L., Koons, C.B. 1983. Oil dispersant field evaluation: IXTOC 1 blowout, Bay of Campeche, Mexico. Oil and Petrochemical Pollution, 1, 183–188.

McKenzie, P., Hughes, D.E. 1976. Microbial degradation of oil and petro-chemicals in the sea. *In* Skinner, F.A., Carr, J.B., ed., Microbiology in agriculture, fisheries and food. Academic Press, New York, NY, USA. 91–108.

MEEE Group. 1986. Addition of hydrocarbon and dispersant Corexit 9527 into marine enclosed experiment ecosystems. *In* Data report of the Marine Ecosystem Experiment Group. Third Institute of Oceanography, Xiamen, People's Republic of China.

Menzel, D.W., Case, J. 1977. Concept and design: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 1–7.

Mironov, O.G., Lanskaya, L.A. 1968. The capacity of survival in seawater polluted with oil products inherent in some marine planktonic and benthoplanktonic algae. Botanicheskii Zhurnal, 53, 661–669.

Morita, R.Y. 1977. The role of microorganisms in the marine environment. *In* Anderson, N.R., Zahuranec, B.J., ed., Oceanic sound scattering prediction. Plenum Press, New York, NY, USA. 445–455.

Ni, C., Zhou, Z, Zheng, H., Li, Z., Xie, J., Zhang, N. 1983. The study of microbial biodegradation of oil pollution in the sea — Part 1: The ecological distribution of oil-degrading bacteria in Xiamen Bay. Acta Oceanologica Sinica, 5, 637–644.

Parsons, T.R., Harrison, P.J., Dovey, H.M., Thompson, P.A., Lalli, C.M., Lee, K., Li, G., Chen, X. 1984a. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 2: Biological effects. Marine Environmental Research, 13, 265–275.

Parsons, T.R., Maita, Y., Lalli, C.M.. 1984b. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, UK. 173 pp.

Peterson, B.J. 1980. Aquatic primary productivity and the ^{14}C-CO_2 method: history of the productivity problem. Annual Review Ecology and Systematics, 11, 359–385.

Topping, G., Windom, H.L. 1977. Biological transport of copper at Lock Ewe and Saanich Inlet: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 135–141.

Wakeham, S.G., Canuel, E.A. 1986. Behaviour of aliphatic hydrocarbons in coastal seawater: mesocosm experiments with [^{14}C] octadecane and [^{14}C] decane. Environmental Science and Technology, 20, 574–580.

Ward, D.M., Atlas, R.M., Boehm, P.D., Calder, J.A. 1980. Microbial biodegradation and chemical evolution of oil from the Amoco spill. Ambio, 9, 277–283.

Wells, P.G. 1984. The toxicity of oil spill dispersants to marine organisms — A current perspective. *In* Allen, T.E., ed., Oil spill chemical dispersants: research, experience and recommendations. American Society for Testing and Materials, Philadelphia, PA, USA. Special Technical Publication 840, 177–202.

Wells, P.G., Abernethy, S., Mackay, D. 1985. Acute toxicity of solvents and surfactants of dispersants to two planktonic crustaceans. *In* Proceedings of the 8th Annual Arctic Marine Oil Spill Program Technical Seminar, 18–20 June 1985, Edmonton, Alberta, Canada. Environment Canada, Ottawa, ON, Canada. 228–240.

Winters, K., O'Donnell, R., Batterton, J.C., Van Baalen, C. 1976. Water soluble components of four fuel oils: chemical characterization and effects on growth of microalgae. Marine Biology, 36, 269–276.

Wong, C.S., Whitney, F.A., Cretney, W.J., Lee, K., McLaughlin, F., Wu, J., Fu, T., Zhuang, D. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 1: Fate of a chemically dispersed crude oil. Marine Environment Research, 13, 247–263.

Zhou, Z., Ni, C., Li, Z., Zheng, H., Xie, J., Zhang, N. 1983. The study of microbial biodegradation of oil pollution in the sea — Part 2: The microbial biodegradation of hydrocarbons. Acta Oceanologica Sinica, 5, 777–782.

Effects of Chemically Dispersed Crude Oil on Marine Phytoplankton: A Comparison Between Two Marine Ecosystem Enclosed Experiments

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Xiamen, People's Republic of China

Two experiments involving the addition of crude oil and dispersant into experimental enclosures were carried out during the Canada–China cooperative marine ecosystem enclosed experiments (MEEE) project. The first experiment (MEEE-83) took place in Saanich Inlet, BC, Canada, and involved the use of Prudhoe Bay crude oil and dispersant Corexit 9527. The second experiment (MEEE-86X) was conducted in Xiamen Bay, People's Republic of China, and involved the use of Shengli crude oil and Corexit 9527. During the Xiamen experiment, the water column was agitated by drastic tidal currents, which promoted dissolution processes and resuspension of settled materials. This changed the fate of the chemically dispersed crude oil compared with results from undisturbed MEEE-83. At the end of the Xiamen experiment, the oil concentration in the seawater was relatively constant. Growth of marine phytoplankton in the Xiamen Bay area could be suppressed when the concentration of oil in the seawater was higher than 1.5 mg·L^{-1}.

In 1986, an experiment (MEEE-86X) was carried out in Wutong Bay, Xiamen, to study the fate of Shengli crude oil in marine enclosures and also the effects of crude oil with dispersant Corexit 9527 on marine ecosystems. A similar experiment (MEEE-83) was conducted in Saanich Inlet, BC, Canada, in 1983. This report compares the results of the two experiments and discusses the effects of both the chemically dispersed crude oil and the dispersant Corexit 9527 on local phytoplankton communities.

Methods

Three plastic bags (2.4 m in diameter × 16 m deep) were used in MEEE-83. Each bag enclosed about 65 m^3 of seawater from the site. The first bag (C1) was used as a control, receiving neither oil nor dispersant. The second bag (C2) was treated with 20 g of Corexit 9527. The third bag (C3) was treated with a mixture of

200 g of Prudhoe Bay crude oil and 20 g of dispersant. Except for during sampling periods, the enclosures remained relatively undisturbed throughout the experiment. At the beginning of the experiment, the enclosures were supplemented with nutrients (N:Si:P = 10:10:1 $\mu g \cdot L^{-1}$) to induce phytoplankton blooms.

In MEEE-86X, several fortified polyethylene bags (2 m in diameter × 6 m deep) were used to enclose about 14 m^3 of seawater. The first bag (C1) was used as the control. The second bag (C2) was treated with 15 g of dispersant Corexit 9527. A mixture of 150 g of Shengli crude oil and 15 g of dispersant Corexit 9527 was added to bag C3. At the beginning of the experiment, nutrients were also added to each enclosure to arrive at initial N:Si:P ratios of 15:15:0.5 $\mu g\text{-}at \cdot L^{-1}$. A phosphate-limiting condition occurred during the experiment because seawater from Xiamen Bay is usually deficient in reactive phosphorus.

Both experiments employed the same sampling methods. Samples were analyzed for the following parameters: water temperature, salinity, nutrient concentrations, chlorophyll *a*, primary productivity, number and species composition of phytoplankton, number and species composition of zooplankton, bacteria and heterotrophic production, settling rate, and concentration of oil components. Analytical methods used in the two experiments were also similar, details of which are described in Parsons et al. (1984a), Wong et al. (1984), and Whitney (1984).

Results

On day 4 of MEEE-83, diatom blooms were observed in bags C1 and C2 (Fig. 1). Species of plankton were similar in these bags throughout the entire experiment. No diatom bloom was observed in bag C3 and abundance of plankton decreased gradually. These observations indicated that diatom growth in bag C3 was significantly suppressed. However, microflagellate growth was fairly conspicuous.

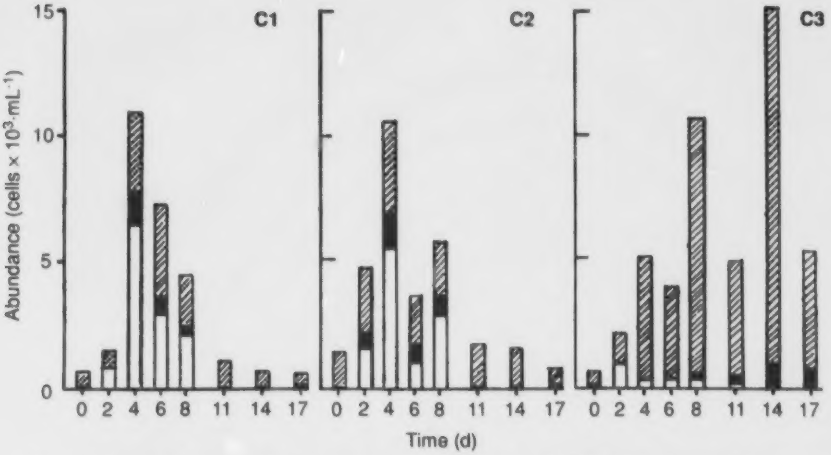

Fig. 1. Changes in centric diatoms (open), pennate diatoms (solid), and microflagellates (shaded) in the three enclosures of MEEE-83.

At the end of the experiment, the abundance of microflagellates reached its maximum, at more than 30 times its initial value. Corresponding with the plankton blooms, amounts of chlorophyll *a* in bags C1 and C2 also reached their peak values

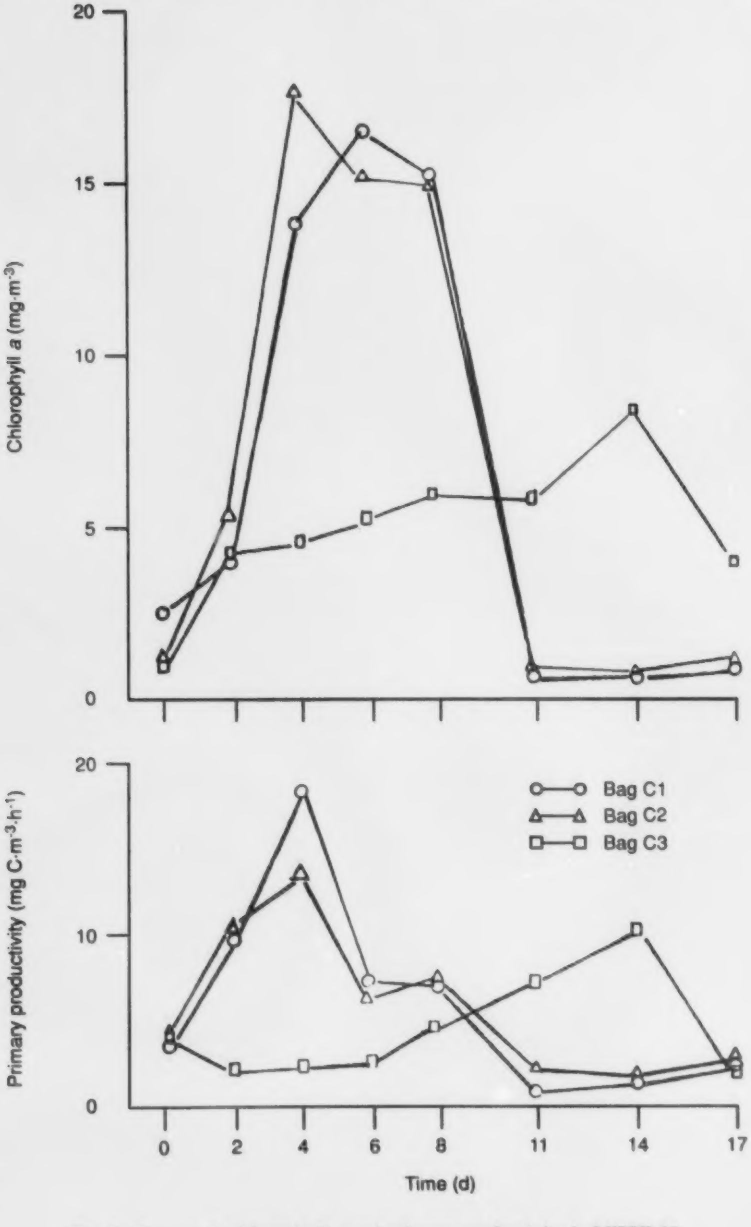

Fig. 2. Changes in chlorophyll *a* and primary productivity in MEEE-83.

on day 4. These amounts decreased to their lowest values after day 11. In bag C3, the amount of chlorophyll *a* remained fairly stable until the end of the experiment when its value increased slightly because of the prolific growth of microflagellates. Trends of primary productivity for bags C1 and C2 paralleled that of chlorophyll *a*, i.e., maximal values were reached on day 4 and minimal values occurred after day 11. In bag C3, the primary productivity decreased at the beginning of the experiment, but rose slightly at the end of the experiment (Fig. 2).

All of the above results indicated that Corexit 9527 alone does not affect the growth of diatoms (Parsons et al. 1984a; Harrison et al. 1986). However, the oil–dispersant mixture in bag C3 suppressed the growth of diatoms, but not the growth of microflagellates. Primary productivity of phytoplankton was inhibited initially by the oil–dispersant mixture, but gradually recovered later.

In MEEE-86X enclosures, the species composition of phytoplankton was basically the same, although the abundance of each species varied over the course of the experiment. In bag C1, a plankton bloom dominated by centric diatoms occurred on day 8, followed by a slight decrease in the number of phytoplankton over the next few days. After day 14, phytoplankton increased rapidly due to tremendous growth of pennate diatoms. Results from bag C2 were similar to those from bag C1, with a plankton bloom occurring between days 2 and 11. No dominant species was observed in bag C3. A small decrease in the total number of phytoplankton in bag C3 occurred after the addition of crude oil, but numbers increased slightly near the end of the experiment.

After the addition of crude oil, the number of centric diatoms decreased significantly, remaining below initial values for the rest of the experiment. However, a small increase in the number of pennate diatoms occurred during the last part of the experiment (Fig. 3). Variations in amounts of chlorophyll *a* in bags C1 and C2 with time followed basically the same trends. In bag C3, after the addition of the oil–dispersant mixture, the amount of chlorophyll *a* decreased rapidly and stayed mostly at a low value. All the results for primary productivity followed the same

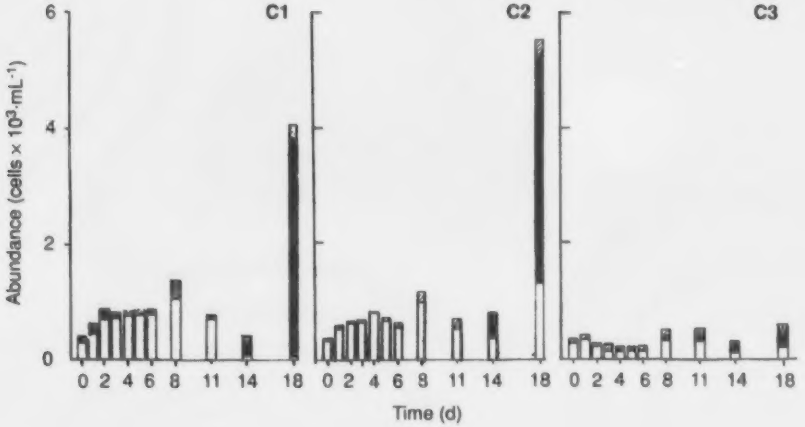

Fig. 3. Changes in centric diatoms (open), pennate diatoms (solid), and microflagellates (shaded) in the three enclosures of MEEE-86X.

trend as chlorophyll *a* with very low productivity in bag C3 throughout the experiment (Fig. 4).

Discussion

Results from MEEE-86X revealed that variations in the abundance of phytoplankton and the amount of chlorophyll *a* were almost identical in the control bag (C1) and the bag treated with dispersant (C2). The structure of the plankton community in each enclosure was fairly stable during the experiment. It can be assumed that the addition of Corexit 9527 to the experimental ecosystems did not affect the structure of the diatom community. This result agreed completely with the results of MEEE-83.

In enclosures treated with chemically dispersed crude oil, suppression of phytoplankton in bag C3 of MEEE-86X was much more severe than that observed in MEEE-83. At the end of the experiment, the abundance of centric diatoms did not recover to its initial value, the number of microflagellates remained low, and there

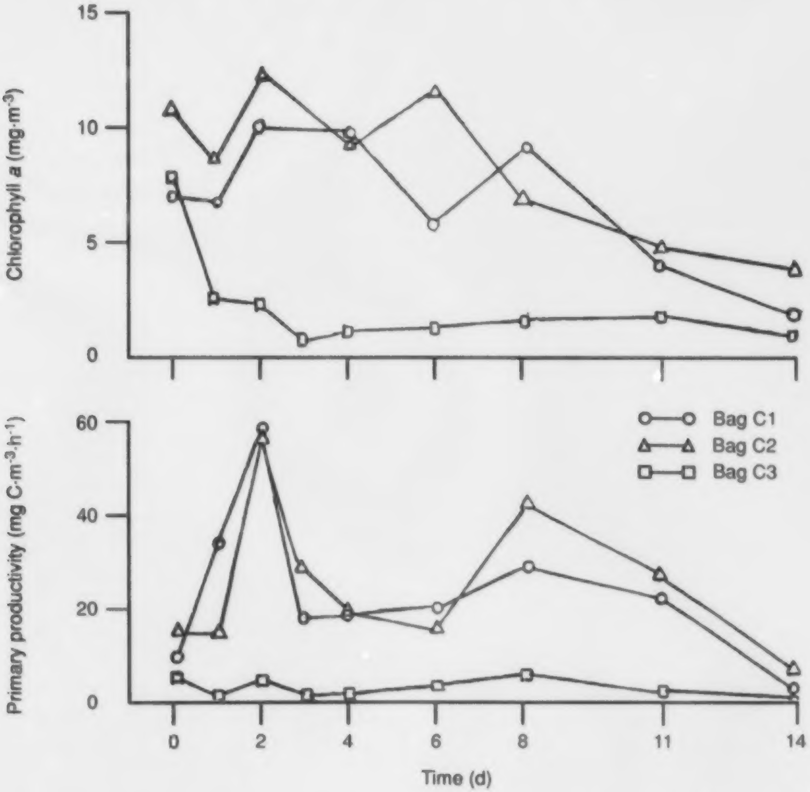

Fig. 4. Changes in chlorophyll *a* and primary productivity in MEEE-86X.

was only a small increase in the number of pennate diatoms; this increase should not, however, be considered as an indication of recovery from oil pollution.

In the later stage of MEEE-83, the number of diatoms increased tremendously, there was a large increase in the number of microflagellates, and primary productivity and amounts of chlorophyll *a* increased. The difference in the degree of plankton suppression between these two experiments was attributed mainly to the total oil concentration (particulate and soluble) in the water column, the concentration in MEEE-86X being much higher than that in MEEE-83. Even at the end of the experiment, the total oil concentration in MEEE-86X was about 1.5 mg·L^{-1}, i.e., about five times the concentration of 0.3 mg·L^{-1} measured in MEEE-83. The quality of the crude oil and the field conditions under which the experiment was conducted probably contributed to the high concentration of oil in the seawater (Fig. 5).

The percentages of nonvolatile alkane in Prudhoe Bay crude oil and Shengli crude oil were 10 and 14% respectively. Total numbers of particles within 3–4 μm diameter, counted before the experimental treatments, were about 20 000 particles·mL^{-1} for both experiments, and the size distribution of the particles was almost identical (Fig. 6). One day after the addition of crude oil in MEEE-83, 51% of the oil was adsorbed on the particles. In the case of MEEE-86X, 80% of the oil was found on the particles and 20% of the total was in soluble form. The crude oil in MEEE-86X was dispersed as an unstable suspension system in the water col-

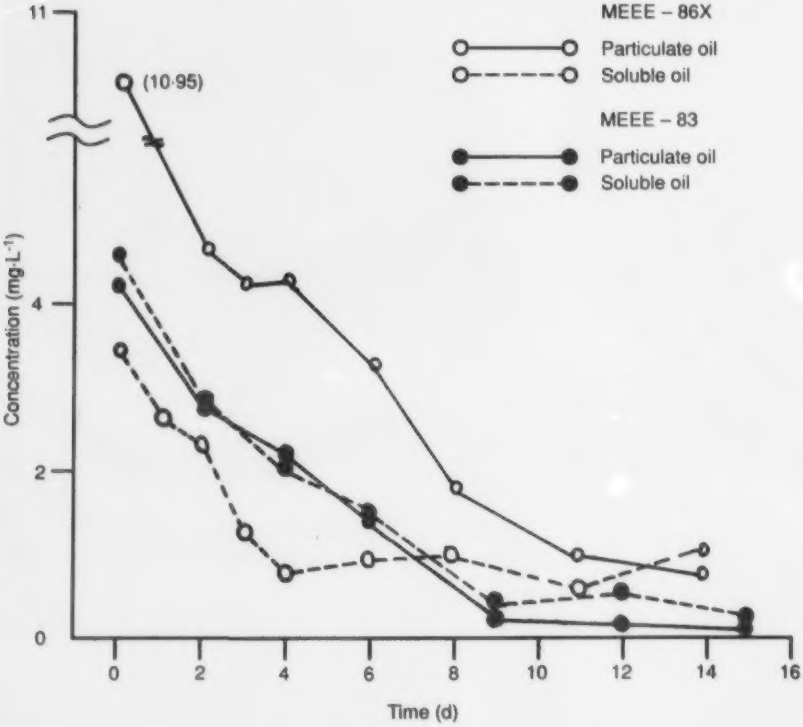

Fig. 5. Oil concentrations.

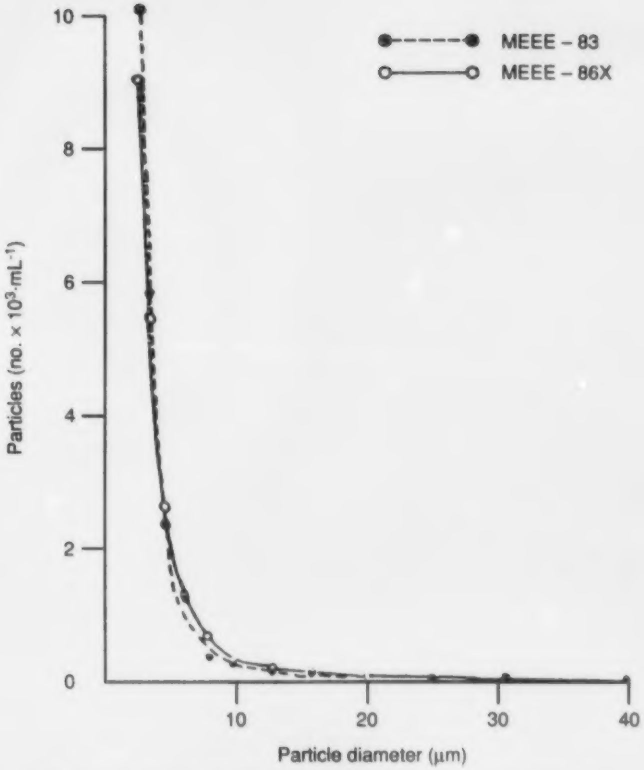

Fig. 6. Particle-size distribution on the day before the addition of oil.

umn. In this system, the concentration of particulate oil could not be lowered quickly. Two weeks later, it was still at a level of 0.85 mg·L^{-1} in contrast to 0.1 mg·L^{-1} in MEEE-83. From day 3 onward, the level of soluble oil remained at about 1 mg·L^{-1} (Fig. 5). Here again, another difference between the two experiments must be taken into account — the water column in the Xiamen enclosures was considerably disturbed.

The Xiamen area experiences semidiurnal tides. The maximum current at the site is about 1.5 knots. Between high and low tides, experimental bags were observed to be bumped around by the tidal currents. These bags were only 6 m in depth and when they were subjected to strong current action, resuspension of settled materials and associated particulate oil in the bags was unavoidable. Results of the n-(1-^{14}C) hexadecane-trace experiment (Fig. 7) also indicated inconsistent radioactivity levels in the water phase. This was most apparent between days 3 and 4 when radioactivity increased from 101 to 498 dpm·L^{-1} with a concomitant decrease in radioactivity in settled materials. On day 4, the settling rate of particulates was significantly reduced.

All of these results indicated the existence of resuspension that minimized the action of sedimentation. In MEEE-86X, the average settling rate of particles after

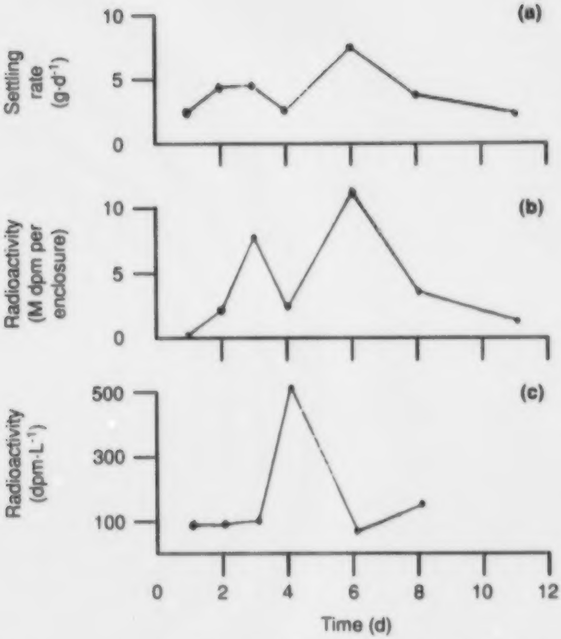

Fig. 7. **(a)** Settling rate of particles; **(b)** ^{14}C in sediment; and **(c)** ^{14}C in seawater.

oil treatment was 3.2 $g \cdot d^{-1}$, with a maximum of 7.3 $g \cdot d^{-1}$; whereas in MEEE-83, the average was 8 $g \cdot d^{-1}$ and the maximum was 24 $g \cdot d^{-1}$. At the end of the experiment, the percentage of settled oil was 14.7% of the total added for MEEE-86X; and 21.4% of the total added for MEEE-83.

One of the important pathways for oil removal is adsorption of oil droplets on plankton or other suspended organic particles followed by their sinking to the bottom to reduce particulate oil in the water column (Lee et al. 1985). In MEEE-86X, oil droplets were also found clinging to the surface of plankton when observed under a fluorescence microscope. Thus, settling of oil adsorbed on suspended organic matter should be occurring in the enclosures. In this experiment, however, the maximum number of phytoplankton was only 600 $cells \cdot mL^{-1}$, which is only 4% of the maximum number in MEEE-83 and even less than the initial abundance (900 $cells \cdot mL^{-1}$) of the latter. The relatively low number of phytoplankton in the enclosures would reduce the chance of the particulate oil sinking. Furthermore, the settling process was also offset by the turbulence-induced resuspension. This reduced even further the already weakened scavenging process.

In MEEE-86X enclosures, those particles adsorbing a high concentration of oil are likely to possess a stronger coalescencing ability than particles absorbing lower amounts of oil. As the enclosed water column is agitated, the chance of collision among the particles increases, which, in turn, enhances the coalescencing ability of the particulate oil. The large particles would then float to the water surface and cling to the inner wall of the bag. Results from MEEE-86X revealed that coalesced particulate oil observed clinging to the inner wall of the bag near the waterline

Table 1. Fate of chemically dispersed crude oil in the MEEE-83 and MEEE-86.

	MEEE-83		MEEE-86X	
	Weight (g)	Percentage of oil added	Weight (g)	Percentage of oil added
Oil in sediment	42.8	21	22.1	15
Oil on bag wall	4.1	2	40.6	27
Particulate oil	5.0	3	8.5	6
Oil in seawater	19.5	10	12.1	8
Others	—	64[a]	—	44[a]

[a] Approximate values.

amounted to 40.6 g or about 27% of the total oil added. In the case of MEEE-83, only 4.1 g of coalesced particulate oil, or 2% of the total oil added, was found clinging to the inner wall of the bag.

Another important phenomenon was observed during MEEE-86X. Three days after the addition of crude oil, the concentration of soluble oil in the water columns leveled off at about 1 $mg \cdot L^{-1}$ (Fig. 5) even with all the transfer and degradation processes in action. This was mainly caused by the high concentration of particulate oil in the water columns, and when the water columns were disturbed, the dissolution process of oil would increase. A laboratory experiment also corroborated that the solubility of crude oil in seawater increases with agitation.

In summary, the disturbed water columns of MEEE-86X enclosures resulted in lower settling action and, therefore, a reduction in oil removal by the scavenging process. At the same time, resuspension of particles enhanced dissolution of particulate oil, resulting in a much higher concentration of oil in the water columns. In other words, disturbing the water in the enclosures altered the transfer and fate of the crude oil in the water columns (Table 1). The biological effect of this alteration is that phytoplankton growth found in the area around Xiamen would be suppressed by dispersed crude oil at a concentration of about 1.5 $mg \cdot L^{-1}$.

Acknowledgments

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Harrison, P.J., Cochlan, W.P., Acreman, J.C., Parsons, T.R., Thompson, P.A., Dovey, H.M., Chen, X. 1986. The effects of crude oil and Corexit 9527 on marine phytoplankton in an experimental enclosure. Marine Environmental Research, 18, 93–109.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parsons, T.R., Lalli, C.M., Wu, J. 1985. Microbial responses to crude oil and Corexit 9527: SEAFLUXES enclosure study. Microbial Ecology, 11, 337–351.

Parsons, T.R., Harrison, P.J., Acreman, J.C., Dovey, H.M., Thompson, P.A., Lalli, C.M., Lee, K., Li, G, Chen, X. 1984a. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 2: Biological effects. Marine Environmental Research, 13, 265–275.

Parsons, T.R., Maita, Y., Lalli, C.M. 1984b. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, NY, USA.

Whitney, F.A., ed. 1984. The effects and fate of chemically dispersed crude oil in a marine ecosystem enclosure — data report and methods. Institute of Ocean Sciences, Department of Fisheries and Oceans, Sidney, BC, Canada. Canada Data Report of Hydrography and Ocean Sciences 29, 77 p.

Wong, C.S., Whitney, F.A., Cretney, W.J., Lee, K., McLaughlin, F., Wu, J., Zhuang, D. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 1: Fate of chemically dispersed crude oil. Marine Environmental Research, 13, 247–263.

Effect of Chemically Dispersed Crude Oil on the Distribution of Primary Microfouling Organisms

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The impact of chemically dispersed crude oil on the formation and distribution of subtropical primary microfouling organisms was studied using marine ecosystem enclosures. Direct microscopic observations showed elevated numbers of bacteria in association with dispersed oil droplets. However, dispersed oil appeared to reduce the formation of the microfilm-slime layer and subsequent growth and attachment processes of macrofouling organisms.

Because oil spills influence physical, chemical, and biological processes in marine ecosystems, they are now recognized as a potential environmental hazard. Although the frequency of marine oil spills is increasing with new offshore developments and greater use of marine transport, little information is available on the effects of oil and oil dispersants on primary microfouling organisms. Thus, during a marine ecosystem enclosed experiment (MEEE) designed to evaluate the environmental impact of a crude oil and an oil dispersant on pelagic primary and secondary producers, changes in the distribution of primary microfouling organisms were also monitored.

Materials and methods

Four marine ecosystem enclosures (MEEs) were launched from a catamaran on 21 May 1986 in Xiamen Bay, People's Republic of China (MEEE Group 1986). The enclosed ecosystems were amended with nutrients (NO_3:SiO_4:PO_4 = 15:10:0.5) immediately after the launch. MEE-1 was designated as a "control" bag; thus it received no further treatment. Using a diffusion apparatus similar to that described by Topping and Windom (1977), 15 g of Corexit 9527 was added homogeneously to the top 3 m of MEE-2. In addition to the dispersant, the same depth zone of MEE-3 was injected with 3.7 MBq of n-(1-^{14}C)-hexadecane and 150 g of Shengli crude oil. Except for the radioisotope addition, MEE-4 was an experimental replicate of MEE-3.

Glass microscope slides were used as the experimental substrate for microfouling organisms. These slides, mounted in a wooden frame, were suspended at 1-m depth within MEE-1, MEE-2, and MEE-3, and at an "open water" site next to the enclosures. The slides were retrieved over a 20-d period. Upon retrieval, they were immediately refrigerated and transported to the laboratory within 2 h for analysis. Slides for microscopic identification and enumeration of bacteria and diatoms were fixed in 3% formalin.

A microscope fitted with epifluorescence and phase-contrast condensers was used for direct observation of oil droplet distribution. Size and morphology of bacteria were observed with a scanning electron microscope using samples that were desalted, dehydrated in ethanol, dried with Freon-13 at the critical point, and coated with gold.

Results and discussion

Formation and succession of microfouling organisms are influenced by a multitude of environmental factors, e.g., nutrient concentrations, temperature, salinity, contaminants, etc. Thus, it is not surprising that numerous species of microfouling organisms were observed during the experiment. After 1 d of immersion, elevated numbers of bacteria (cocci, bacilli, and vibrio) were found in association with dispersed oil droplets adhering to the surface of slides recovered from MEE-3 and MEE-4. Morphologically, these bacteria did not differ from indigenous microorganisms found on surfaces of other submerged objects recovered from the waters of Xiamen Bay. In addition to bacteria, microfouling organisms, including fungi, yeasts, and diatoms (Table 1), were also observed.

Bacteria, such as *Achromobacterium*, *Pseudomonas*, *Vibrio*, and *Acinebacter*, isolated during the experiment are known to have the potential to degrade hydrocarbons. The ability of yeasts and fungi to use hydrocarbons as sole sources of carbon and energy is a well-documented phenomenon and their isolation from oil-contaminated environments has suggested that they also play an important role in degradating oil spilled in the environment (Nyes et al. 1968; Ahearn and Meyers 1972; Cerniglia and Perry 1973). The primary colonizing species found in this study have frequently been isolated from submerged substrates found elsewhere (Corpe 1972; Walker and Colwell 1976). The predominant microfouling bacteria, *Pseudomonas* spp, are generally reported in experiments using liquid culture medium designed to isolate oil-degrading bacteria (Austin et al. 1977; Riquelme and Garcia-Tello 1986), and are known to have potentially high degradation rates for crude oil and its components (Cundell and Traxler 1973; Walker and Colwell 1976; Austin et al. 1977).

Colonization by filamentous bacteria, diatoms, and barnacles on glass slides recovered from MEE-3 and MEE-4 on day 4 was patchy and incomplete. In comparison with slides immersed in "open water" and in the "control" enclosure, it was evident that bacterial in the treated bags was stimulated by the dispersed oil. Bacteria that have the capacity to produce extracellular polysaccharides are generally the first microfouling organisms to inhabit the surface of submerged substrates, forming a slime layer in a matter of days.

Laboratory experiments with batch cultures have demonstrated that the proportion of bacteria that adhere to the walls of the culture vessel increases as the

Table 1. Bacteria, filamentous fungi, yeasts, and diatoms observed on the surface of glass slides recovered from MEEE enclosures.

Bacteria

- *Achromobacter* spp
- *Acinebacter* spp
- *Pseudomonas* spp
- *Vibrio* spp

Yeasts

- *Candida* spp
- *Citermyces* spp
- *Cryptococcus* spp
- *Schizosaccharomyces* spp

Fungi

- *Aspergillus* spp
- *Clodosporium* spp
- *Dendryphion* spp
- *Mecelia sterilla*
- *Penicillus* spp
- *Torula* spp

Diatoms

- *Achnanthes brevipes*
- *Amphora angusta*
- *Amphora coffaeiformis*
- *Bacteriastrum* spp
- *Biddulphia mobiliensis*
- *Biddulphia reticulata*
- *Caloneis formosa*
- *Cocconeis pellucida*
- *Cocconeis heteroidea*
- *Cyrisigma balticum*
- *Diploneis bombus*
- *Diploneis incurvata*
- *Fleueosigma naviculaeum*
- *Fleueosigma rhombeum*
- *Grammatophora marine*
- *Licmophora flabellata*
- *Mastogloia fascistriata*
- *Melosira nummuloides*
- *Navicula comcellata*
- *Navicula marine*
- *Nitzschia obtusae*
- *Nitzschia paleacea*
- *Nitzschia panduriformis*
- *Rhizosolenia styliformis*
- *Stauroneis constricta*
- *Trachyneis aspera*

numbers of free bacteria in solution increase. Stimulation of bacterial growth by Corexit-dispersed crude oil has also been demonstrated in marine mesocosm studies conducted by Lee et al. (1985). In the present experiment, between days 2 and 6, enhanced growth of unattached and primary microfouling bacteria due to Corexit-dispersed oil was observed simultaneously on the glass slides. Furthermore, observations of the close association of bacteria and oil droplets suggest a positive chemotaxic response by bacteria to the oil or rapid growth by the oil-degrading bacteria, or both.

Nutrient limitation by nitrogen and phosphorus has been recognized as a major factor controlling bacterial growth in the marine environment, especially in situations where carbon is in great excess after an oil spill. Stimulation of bacterial growth in the present experiment may be attributed to nutrient enrichment and dispersive action by Corexit 9527, which decreases clumping of cells, thus maximizing the surface area available for nutrient absorption. Although zooplankton grazing may have a significant effect on the development of microbial microfouling communities, this process was not assessed during the present experiment.

A large number of benthic and attached diatoms frequently found in the waters of Xiamen Bay were observed on the surfaces of the recovered glass slides (Table 2). Although the close association between the distribution of diatoms and

Table 2. Numbers of major macrofouling organisms observed on the surface of glass slides recovered after 12 d of exposure.

Species	Open water	MEE-1	MEE-3	MEE-4
Tubularia spp	4	1	0	0
Oystrea spp	3	1	0	0
Balanus spp	3	1	0	0

oil droplets observed under phase-contrast microscopy in the present experiment could be regarded as a chemotaxic response of diatoms to oil, it could also be attributed to physicochemical interactions between diatom surface polysaccharides and dispersed oil. Results obtained in arctic waters by Hsiao (1978) suggest that oil spills would result in phytoplankton communities dominated by flagellates because of the sensitivity of diatoms to oil. In contrast to these results, stimulatory effects of dispersed crude oil on the growth of bacteria (Fig. 1) and diatoms (Fig. 2) were observed in our experiment. Unlike bacteria (Fig. 1), however, an immediate stimulatory response by diatoms to dispersed oil was not observed on day 1 (Fig. 2).

Previous experiments on the ecology of microfouling organisms in Xiamen Bay demonstrated a two-tier fouling phenomenon in which the organisms form a dis-

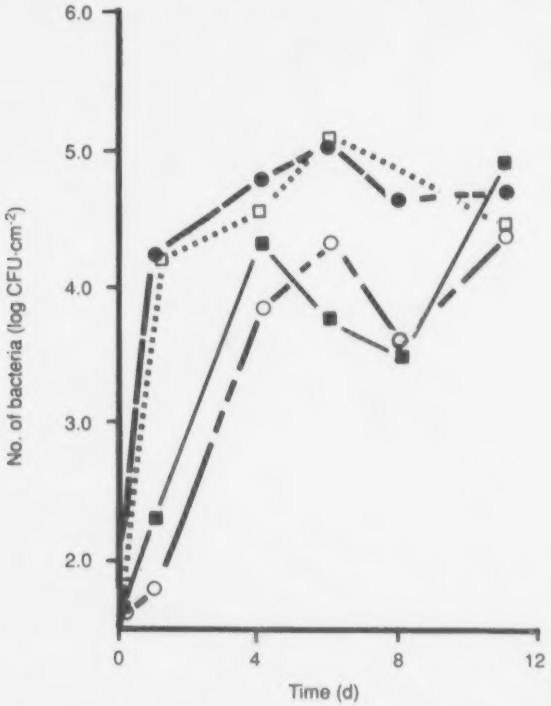

Fig. 1. Heterotrophic bacteria observed on the surface of glass slides recovered from MEE-1, MEE-3, MEE-4, and "open-water."

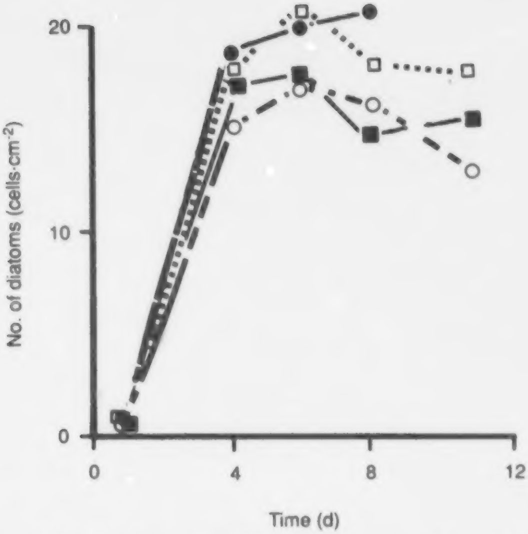

Fig. 2. Diatoms attached to the surface of glass slides recovered from MEE-1, MEE-3, MEE-4, and "open water."

tinct microfilm-slime layer within 7 d. In the present experiment, formation of such a layer was observed on slides immersed in the "open water" after 7 d. Because of seasonal and geographic variations, however, the observed slime layer was not as thick as expected. Microfilm formation was slower on the slides recovered from MEE-3 and MEE-4 compared with that on slides recovered from MEE-1 and the "open water." Extensive development of a slime layer was not observed on slides exposed to dispersed oil because of physiochemical alterations of the surface microlayer or growth inhibition induced by the Corexit-dispersed oil.

Zobell and Allen (1935) noted that bacteria and, to a lesser extent, other microorganisms (fungi, diatoms, etc.) are the organisms that colonize submerged surfaces during the early stage, thereby forming a primary film. They are followed by macroscopic organisms that belong to the primary successional stages. In the present experiment, after 4 d of immersion in "open-water" conditions, macrofouling organisms (predominantly bryozoans) covered 10–15% of the surface area of the glass slides, greatly impeding further development of microfouling films. After 7 d, species found on the slides included barnacles and oysters. The numbers and diversity of macrofouling organisms were much greater on slides immersed in "open water" than those immersed in the "control" enclosure (Table 2). In contrast, larval and adult forms of macrofouling organisms were not observed on the slides suspended in MEE-3 or MEE-4 during the experiment, suggesting that dispersed oil inhibited the growth or attachment mechanism, or both, of the macrofouling organisms.

In many aquatic ecosystems, particularly when the water is low in nutrients, surfaces colonized by microorganisms are sites of relatively intense biological activity. Consortia composed of attached microalgal and bacterial populations have essential roles in aquatic ecosystems, acting as sites of active nutrient regeneration (Loeb and Rueter 1981; Rueter et al. 1983) and serving as a trophic resource for other organisms (Cattaneo 1983). Attached biofilm communities are also relevant to industry, because microbial fouling is associated with corrosion and reduces the efficiency of industrial processes (Characklis and Cooksey 1983).

With the expected increase in the incidence of oil spills associated with coastal and offshore developments globally, it is evident that further studies on these processes should be conducted.

Acknowledgments

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Ahearn, D.G., Meyers, P. 1972. The role of fungi in the decomposition of hydrocarbons in the marine environment. *In* Walters, A.H., Heuckvander, H. ed., Biodeterioration of materials. Applied Science Publishers, London, UK. 12–18.

Austin, B., Calomoris, J.J., Walker, J.D., Colwell, R.R. 1977. Numerical taxonomy and ecology of petroleum-degrading bacteria. Applied and Environment Microbiology, 34, 60–68.

Cattaneo, A. 1983. Grazing on epiphytes. Limnology and Oceanography, 28, 124–132.

Cerniglia, C.E., Perry, J.J. 1973. Crude oil degradation by microorganisms isolated from the marine environment. Zeitschrift fuer Allgemeine Microbiologie, 13, 299–306.

Characklis, W.G., Cooksey, K.E. 1983. Biofilms and microbial fouling. Advances in Applied Microbiology, 29, 93–138.

Corpe, W.A. 1972. Microfouling: the role of primary film forming marine bacteria. *In* Proceedings of the 3rd International Congress on Marine Corrosion Fouling. Northwestern University Press, Evanston, IL, USA.

Cundell, A.M., Traxler, R.W. 1973. The isolation and characterization of hydrocarbon utilizing bacteria from Chedabucto Bay, Nova Scotia. *In* Proceedings of a joint conference on prevention and control of oil spills; 13–15 March 1973, Washington, DC, USA. American Petroleum Institute, Washington, DC, USA. 421–426.

Hsiao, S.I.C. 1978. Effects of crude oils on the growth of arctic marine phytoplankton. Environmental Pollution, 17, 93–107.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parsons, T.R., Lalli, C.M., Wu, J. 1985. Microbial response to crude oil and Corexit 9527: SEAFLUXES enclosure study. Microbial Ecology, 11, 337–351.

Loeb, S.L., Rueter, J.E. 1981. The epilithic periphyton community: a five lake comparative study of community productivity, nitrogen metabolism and depth distribution of standing crop. Internationale Vereinigung für Theoretische und Angewandte Limnologie, Verhandlugen, 21, 346–352.

MEEE Group. 1986. Addition of hydrocarbons and dispersant Corexit 9527 into marine enclosed experimental ecosystems. *In* Data report of the Marine Ecosystem Enclosure Experiment Group. Third Institute of Oceanography, Xiamen, People's Republic of China. Unpublished.

Nyes, E.J., Auquieseaud, J.P., Wiaux, L. 1968. Taxonomic value of the property of fungi to assimilate hydrocarbons. Antonies van Leeuwenhoek, 34, 441–457.

Riquelme, C.E., Garcia-Tello, P. 1986. Effects of oil contamination on colonization of marine periphytic microflora. Journal of the Oceanographical Society of Japan, 42, 294–299.

Rueter, J.E., Loeb, S.L., Goldman, S.R. 1983. Nitrogen fixation in periphyton of oligotrophic Lake Tahoe. *In* Wetzel, R.G., ed., Periphyton of freshwater ecosystems. W. Junk Publishing, Amsterdam, Netherlands. 101–109.

Topping, G., Windom, H.L. 1977. Biological transport of copper at Loch Ewe and Saanich Inlet: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 135–141.

Walker, J.D., Colwell, R.R. 1976. Enumeration of petroleum-degrading microorganisms. Applied and Environmental Microbiology, 31, 198–207.

Zobell, C.E., Allen, E.C. 1935. The significance of marine bacteria in the fouling of submerged surfaces. Journal of Bacteriology, 29, 230–251.

Effects of Shengli Crude Oil and Dispersant Corexit 9527 on Zooplankton in Marine Ecosystem Enclosed Experiments

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A marine ecosystem enclosed experiment was carried out by Chinese and Canadian scientists in the eastern part of Xiamen Bay (24° 32′ 30″ N, 118° 11′ 18″ E) during a 3-week period in May and June 1986. The purpose of the experiment was to assess the chemical actions of Shengli crude oil and Corexit 9527 and their ecological effects in the enclosures. Particular attention was paid to the effects of the two chemicals on zooplankton communities in the experimental enclosures. The main conclusions were: first, that Corexit 9527 alone inhibited the development of herbivorous copepods to some extent, but that the population could recover and develop; however, carnivorous copepods and other groups of zooplankton did not exhibit a similar response. Second, Corexit 9527 mixed with Shengli crude oil caused a rapid reduction in all zooplankton phyla populations. These results were compared with a similar experiment conducted in Saanich Inlet, BC, Canada, in July 1983.

In recent years, many countries have employed marine experimental ecosystem enclosures to study the effects of crude oil, dispersants, and mixtures of the two on resident populations. For example, Elmgren and Frithsen (1982) and Dahl et al. (1983) conducted medium-scale enclosure experiments with additions of pollutants and reported the effects of these pollutants on zooplankton. Because concentrations of crude oil, and especially dispersant, used in earlier experiments were often too high and bioassays were conducted on only single species, a more realistic experiment was designed. The experiment was fashioned after a similar marine ecosystem enclosed experiment (MEEE) carried out in Saanich Inlet, BC, Canada, in 1983 (MEEE-83-Saanich).

This paper reports on the effects of Corexit 9527 dispersant alone and mixed with oil on the growth and development of zooplankton populations in enclosed experimental ecosystems.

Methods

During a 3-week period in May and June 1986, Chinese and Canadian scientists conducted an in-situ experiment in the eastern part of Xiamen Bay (24° 32′ 30″ N, 118° 11′ 18″ E) (MEEE-86-Xiamen). The enclosures consisted of four bags (referred to as bags 1, 2, 3, and 4) that were 2 m in diameter × 6 m long, and enclosed about 14 m^3 of seawater each. The bags were supported from a catamaran. The four bags were treated as follows: bag 1 was the control, receiving no pollutants; bag 2 received 15 g of Corexit 9527 dispersant for a final concentration of 1.5 $mg \cdot L^{-1}$; bag 3 received 150 g of Shengli crude oil and 15 g of Corexit 9527 dispersant for final concentrations of 15 $mg \cdot L^{-1}$ and 1.5 $mg \cdot L^{-1}$, respectively; and bag 4 was a duplicate of bag 3.

Methods used to analyze zooplankton populations were similar to those used in MEEE-83-Saanich and MEEE-85-Xiamen. Zooplankton samples were collected vertically from the bottom to the surface in the centre of the bags between 0900 and 1000 hours using a nylon net 21.5 cm in diameter × 70 cm in length, with a mesh size of 202 µm. Formalin (4 mL and containing <36% formaldehyde) was added to preserve zooplankton samples that had been concentrated to 96 mL.

Zooplankton in the samples were identified and counted under a dissecting microscope. In all, 40 samples were collected, most of which contained limited numbers of zooplankton; therefore, all zooplankton, in the samples were counted. A few samples, which contained many zooplankton, were divided with a Folsom splitter before counting, and one-quarter or one-half of the zooplankton were counted. The counts were then converted by multiplication to numbers per sample and per tow. Abundance of zooplankton populations was expressed as the number of individuals per cubic metre.

Results

Copepods were always dominant among the zooplankton in all four bags. *Acartia pacifica*, *Paracalanus parvus*, *Centropaqes tenuiremis*, *Oithona similis*, and *Temora turbinata* were classified as herbivorous copepods; *Corycaeus affinis* was considered a carnivorous species. Unidentified copepod larvae and nauplii were not included in the counts. The four enclosed ecosystems also contained *Oikopleura* sp., medusae, *Sagitta* sp., *Evadne tergestina*, ostracoda, amphipoda, and meroplanktonic larvae of benthic worms.

These zooplankton, especially the copepods, showed distinct temporal changes. Therefore, for the purpose of this paper, the experimental period has been divided into an early stage, (23–31 May), a middle stage, (31 May–6 June), and a late stage, (6–10 June). The relative abundance of various zooplankton groups is described for each stage.

Control bag

The dominant species was *Paracalanus parvus*, constituting 88% of the total number of zooplankton..However, as shown in Fig. 1, herbivorous copepods were not very abundant during the early stage, with an initial abundance of 1 270 $individuals \cdot m^{-3}$. During the early stage, the abundance of herbivorous copepods was variable, but tended to decrease, averaging 950 $individuals \cdot m^{-3}$. During the middle stage, numbers increased rapidly to reach a peak of 4 300 $individuals \cdot m^{-3}$ on 3 June before declining rapidly to 1 510 $individuals \cdot m^{-3}$. The average abundance of herbivorous copepods during this stage was 2 100 $individuals \cdot m^{-3}$. During the

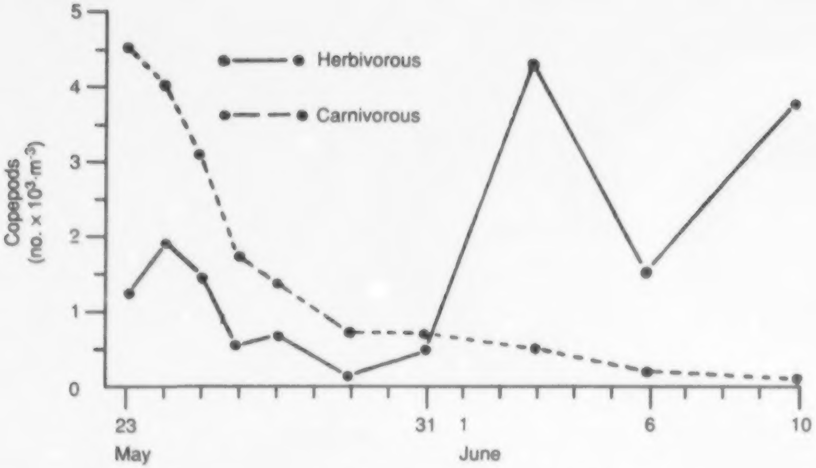

Fig. 1. Change in the abundance of copepods in bag 1.

late stage, numbers again increased, reaching 3 730 individuals·m^{-3} and averaging 2 620 individuals·m^{-3}.

Initially, carnivorous copepods were 3.6 times more abundant than herbivorous species, with an initial density of 4 560 individuals·m^{-3}; however, they declined greatly during the early stage to a low of 720 individuals·m^{-3}, averaging 2 350 individuals·m^{-3} over the period. The decreasing trend continued during the middle stage, but the rate of decline was slower, reaching a low of 240 individuals·m^{-3} and averaging 500 individuals·m^{-3}. The decline continued throughout the late stage with a low of 140 individuals·m^{-3} and an average of 190 individuals·m^{-3}.

Polychaete larvae were present in numbers averaging 4 930, 480, and 170 individuals·m^{-3} during the early, middle, and late stages respectively. Cladocera averaged 410 individuals·m^{-3} during the early stage, but declined to zero in the middle and late stages. Other zooplankton groups were few in number and, in general, decreased even further or eventually disappeared.

Dispersant-treated bag

Paracalanus parvus was also dominant in this bag, forming 78% of the total zooplankton. As shown in Fig. 2, the abundance and change in density of herbivorous copepods during the early stage was similar to that observed in bag 1 with an initial abundance of 1 920 individuals·m^{-3}, but a lower average abundance of 730 individuals·m^{-3}. Numbers increased during the middle stage, but the magnitude was not great and did not form a peak, the maximum being 940 individuals·m^{-3}, and the average being 650 individuals·m^{-3}, which was only one-third that of the average calculated for bag 1 over the same period. During the late stage, numbers increased faster and reached a maximum of 2 380 individuals·m^{-3} by the end of the experiment; the average was 1 660 individuals·m^{-3}. As in bag 1, the numbers of carnivorous copepods declined throughout the experiment. The minimum and average

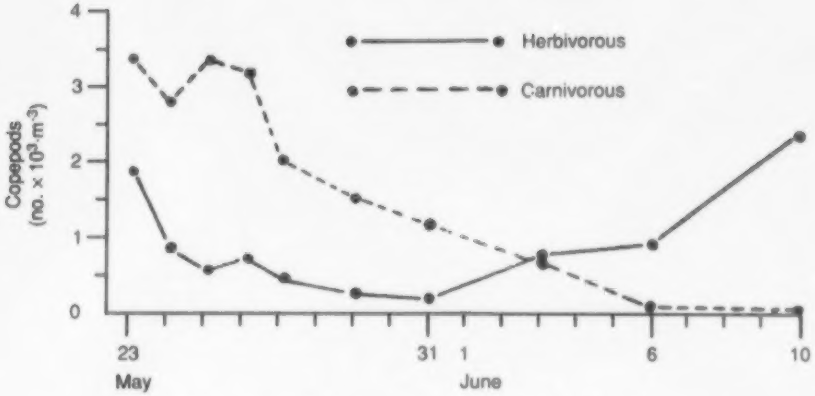

Fig. 2. Change in the abundance of copepods in bag 2.

abundances for the early, middle, and late stages were 1 200 and 2 510, 100 and 660, and 80 and 90 individuals·m^{-3}, respectively.

Other zooplankton groups less dominant than copepods were similar to those observed in bag 1. Average abundances of polychaete larvae and Cladocera during the early, middle, and late stages were 5 100 and 930, 1 020 and 90, and 220 and 40 individuals·m^{-3}, respectively.

Oil- and dispersant-treated bags

In bag 3, *Paracalanus parvus* was again dominant, constituting 82% of the total amount of zooplankton. Figure 3 clearly shows the rapid decrease in the density of herbivorous copepods during the early stage, especially during the first 3 d, from an initial abundance of 880 individuals·m^{-3} to 70 individuals·m^{-3}, with an average abundance of 290 individuals·m^{-3}. Numbers of herbivorous copepods remained very low throughout the remainder of the experiment, with average abundances of 10 and 50 individuals·m^{-3} during the middle and late stages, respectively. The initial abundance of carnivorous copepods was 3 100 individuals·m^{-3}, or 3.5 times that of the herbivorous species. Carnivorous species decreased in a manner similar to that observed for the herbivores. Average abundances for the early, middle, and late stages were 600, 20, and 10 individuals·m^{-3}, respectively.

Average abundances of polychaete larvae in bag 3 during the early, middle, and late stages were 1 060, 300, and 220 individuals·m^{-3}, respectively. Cladocera had an initial abundance of 260 individuals·m^{-3}, but decreased to zero in the later stages. Other zooplankton groups were similar to those observed in bags 1 and 2 in terms of their low densities and temporal changes.

Bag 4 was a duplicate of bag 3 and changing trends in abundance were almost the same in both bags (Fig. 4). *Paracalanus parvus* was again the dominant species. The initial abundance of herbivorous copepods was 820 individuals·m^{-3}. Average abundances during the early, middle, and late stages were 380, 30, and 60 individuals·m^{-3}, respectively. The initial abundance of carnivorous copepods was 3 400 individuals·m^{-3}, or four times that of the herbivorous species. Average

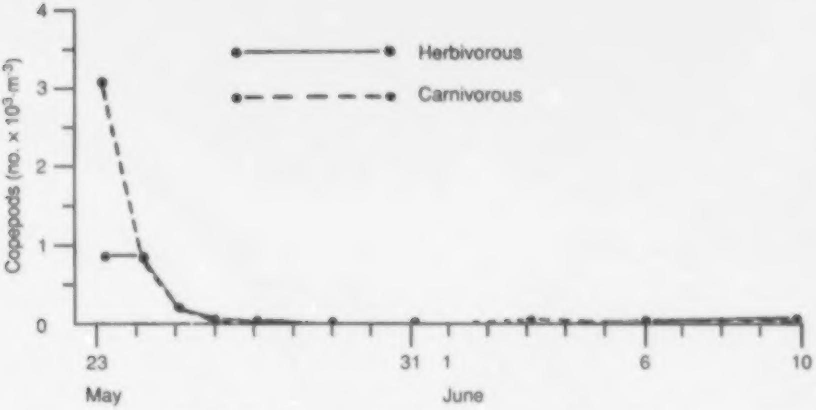

Fig. 3. Change in the abundance of copepods in bag 3.

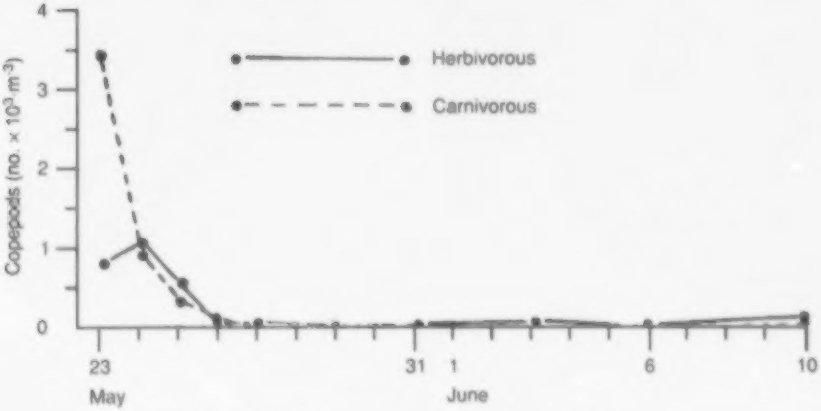

Fig. 4. Change in the abundance of copepods in bag 4.

abundances of carnivores during the early, middle, and late stages were 680, 10 and 20 individuals·m^{-3}, respectively.

Cladocera in bag 4 were present during the early stage at a density of 90 individuals·m^{-3}, but declined to zero after a few days. Polychaete larvae had densities of 890, 80, and 110 individuals·m^{-3} during the early, middle, and late stages, respectively.

Discussion and conclusions

The enclosed seawater columns in all four experimental bags were part of the same seawater body and had the same initial ecosystem structure. This conclusion is based on measurements of the bacterial biomass, bacterial productivity, phytoplankton biomass, chlorophyll *a* concentration, primary productivity, and seawater chemistry (see *MEEE 86-2. Dispersions of crude oils in sea water, October 3–24, 1986*. Unpublished, 28 pp.). It is also supported by initial measurements of various parameters of zooplankton community structure, including species composition of herbivorous and carnivorous copepods, initial abundance of the dominant species, and initial relative abundance of other zooplankton species.

Within the first 3 d, phytoplankton in the control bag (bag 1) reached a peak (Fig. 5). Although the amount of phytoplankton varied afterward, the average chlorophyll *a* concentration during the early stage reached 7.2 mg·m^{-3}. With the consumption of nutrients (nitrate and silicate) and feeding by herbivores, the amount of phytoplankton decreased rapidly; thus, the average chlorophyll *a* concentration was only 5.2 mg·m^{-3} by the middle stage. This coincided with herbivorous copepods reaching their maximal density. These results agree with naturally occurring processes and are similar to results derived from similar experiments (see Parsons et al. 1984).

In bag 2, Corexit 9527 dispersant had no obvious effects on the growth and development of carnivorous copepods, but it did depress numbers of herbivorous copepods. However, the herbivore population recovered within several days to a density about one-third less than that observed in the control at the same stage. This result contrasts with MEEE-83-Saanich (Parsons et al. 1984), which demonstrated that the addition of Corexit 9527 alone did not depress herbivorous copepod numbers relative to the control. The difference in results is probably attributable to the higher concentration of Corexit 9527 used in the Xiamen experiment (1.5 mg·L^{-1}), being about five times that used in the 1983 experiment (0.3 mg·L^{-1}). The average chlorophyll *a* concentration (5.6 mg·m^{-3}) in bag 2 was similar to that measured in the control bag during the middle stage (Fig. 5). Therefore, depression of herbivorous copepod numbers during this period was not related to the biomass of phytoplankton available as food.

The mixture of crude oil and Corexit 9527 added to bags 3 and 4 had a great inhibitive effect on the development of herbivorous and carnivorous copepod

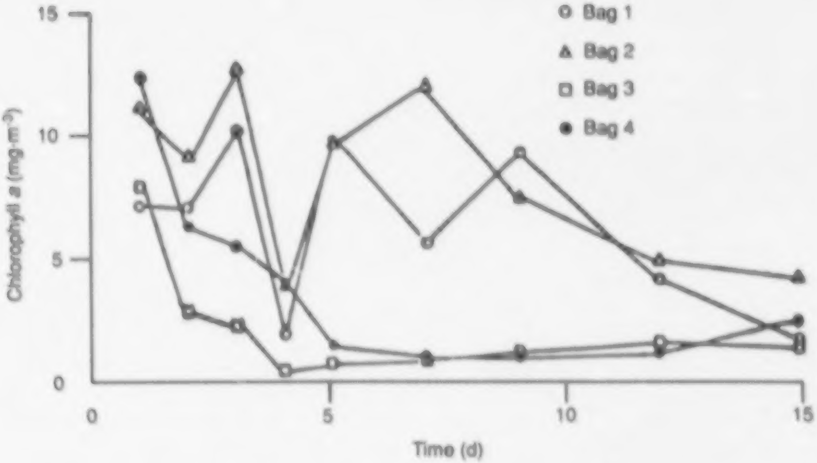

Fig. 5. Change in chlorophyll *a* concentration in bags 1, 2, 3, and 4.

populations, both decreasing rapidly and neither recovering afterward. Crude oil in seawater is present in two forms of emulsions: water wrapped in oil and oil wrapped in water — only a small amount of oil is actually dissolved in the seawater. The presence of the dispersant cannot promote dissolution of various hydrocarbons in seawater, but it accelerates dispersion of crude oil. The population abundance of herbivorous and carnivorous copepods decreased greatly during the first 3 d (23–26 May) (Figs 3 and 4). Experimental data show that the oil content (including oil particles and oil in seawater) on the 1st d was 21.73 $mg \cdot L^{-1}$, and that the average over the first 3 d was 11.94 $mg \cdot L^{-1}$. Later, the average decreased to 3.02 $mg \cdot L^{-1}$. However, copepods did not respond to the decrease in oil content.

The phytoplankton populations in bags 3 and 4 were also depressed by the addition of the oil-dispersant mixture. In bag 3, chlorophyll *a* decreased from an initial concentration of 7.8 $mg \cdot m^{-3}$ to 0.5 $mg \cdot m^{-3}$ and did not recover later. Therefore, the decline in food may have been responsible, at least in part, for the depression of copepod numbers. Similarly, the decrease in the number of carnivorous copepods in bags 3 and 4 would be linked with decreases in the numbers of herbivorous copepods, which are their food.

Zooplankton other than copepods did not seem to respond to Corexit 9527 alone, but they showed similar depressive responses to the mixture of Corexit 9527 and crude oil, and their growth and development were seriously inhibited. This conclusion is based on the changes in the amounts of polychaete larvae and Cladocera. All other zooplankton showed a similar trend, although their abundances were very low throughout the experiment.

Acknowledgment

I wish to acknowledge the State Oceanic Administration of China and the International Development Research Centre of Canada for their cooperative support.

Dahl, E., Laake, M., Tjessem, K., Eberlein, K., Bohle, B. 1983. Effects of Ekofisk crude oil on an enclosed planktonic ecosystem. Marine Ecology Progress Series, 14, 81–91.

Elmgren, R., Frithsen, J.B. 1982. The use of experimental ecosystems for evaluating the environmental impact of pollutants: a comparison of an oil spill in the Baltic Sea and two long-term, low- level oil addition experiments in mesocosms. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 153–165.

Parsons, T.R., Harrison, P.J., Acreman, J.C., Dovey, H.M., Thompson, P.A., Lalli, C.M., Lee, K., Li, G., Chen, X. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 2: Biological effects. Marine Environmental Research, 13, 265–275.

Fate of Chemically Dispersed Shengli Crude Oil in a Marine Ecosystem Enclosure

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The fate of crude oil dispersed with Corexit 9527 was studied in a marine ecosystem enclosure. Chemically dispersed crude oil from Shengli, China, was added to the upper 3 m of the water column at an initial concentration of 15 $mg \cdot L^{-1}$*. Time-series observations were made during the 14-d experiment. The quantity of oil in the enclosure during each phase was determined using a gravimetric method. Results showed that the crude oil–dispersant mixture in the water column was quickly distributed between the filter-passing and non-filterable particulate phases by diffusive and convective processes. It was observed that the oil settled down to the sediment gradually with sinking organic materials, but 27% of the oil added became coated onto the wall of the bag. Resuspension of oil into the water column was also observed.*

In recent years, scientists have been interested in the fate of crude oil and its derivatives in the marine environment and their effects on marine ecosystems. Many marine chemists and marine ecologists consider studies of marine enclosed ecosystems as a bridge (Lacaze 1974; Lee et al. 1977; Davies et al. 1980; Dahl et al. 1983) to understanding discrepancies between results obtained in the laboratory and in natural ecosystems. Cretney et al. (1981) reported on biodegradation of Prudhoe Bay crude oil dispersed by Corexit 9527 in a marine ecosystem enclosure experiment (MEEE). In their experiment, the oil–dispersant mixture was added into the surface waters of the enclosed ecosystem.

In 1983, the fate of Prudhoe Bay crude oil dispersed by Corexit 9527 and its effect on an enclosed ecosystem in Saanich Inlet, BC, Canada, was also studied (Parsons et al. 1984a; Wong et al. 1984; Lee et al. 1985; Harrison et al. 1986). In this study, the mixture was carefully added into the subsurface waters (2–4 m depth) to minimize coalescence of dispersed oil particles at the air–sea interface and to reduce surface evaporative loss of the lighter fractions. These studies have allowed a better understanding of the effects and behaviour of oil–dispersant mixtures injected into ecosystems by different methods.

Another MEEE was carried out in Xiamen, People's Republic of China, in 1986. A modified catamaran, developed by the Institute of Ocean Sciences in Sydney, BC, Canada, originally used for pollution research on marine enclosed ecosystems (Wong et al., this volume), was used for this study to overcome rough sea conditions. In this study, experimental bags made of polyethylene were filled with natural seawater on site. A mixture of crude oil from the Shengli oil field, People's Republic of China, and Corexit 9527 was added to the upper 3 m to study the transfer, fate, and ecological response of dispersed oil.

The experiments carried out in Saanich Inlet in 1983 and in Xiamen in 1986 differ in that the former used an enclosed water body of 65 m^3 and a Prudhoe Bay crude oil–Corexit 9527 dispersant mixture introduced at 2–4 m depth and the latter used a water body of 13 m^3 and a Shengli crude oil–Corexit 9527 dispersant mixture introduced at 0–3 m depth. Also, the Xiamen site more closely represents an open-ocean situation with a strong current of 1 $m \cdot s^{-1}$. The site may be useful in establishing correlations between laboratory and open-water studies and in supplying better scientific data for marine environmental protection.

Methods

Experimental design

The steel catamaran, 12.4 m × 3.6 m, was anchored in the eastern part of Xiamen Bay (24° 32′ 30″ N, 118° 11′ 18″ E), where the strongest current is about 1 $m \cdot s^{-1}$ and the water depth is 15–20 m. Each of the four double-layered polyethylene bags was 6 m long × 2 m in diameter and was fitted with a sediment collector at the deep end. When the sea was relatively calm on 21 May 1986, the bags were lowered to about 5 m depth and were then pulled up to the surface and attached to the catamaran as quickly as possible. About 13 m^3, 95–100% of the volume of the bag, of seawater was enclosed in each bag. On the same day that the bags were launched, nutrients were added to the upper 3 m of the surface water. Concentrations of inorganic nitrate, silicate, and phosphate were 15, 10, and 3 $\mu g \cdot L^{-1}$, respectively. Bag 1 was used as a control (neither dispersant nor oil were added), bag 2 was used as a dispersant bag (15 g of Corexit 9527 was injected at 0–3 m depth to achieve an initial dispersant concentration of about 1.5 $mg \cdot L^{-1}$), and bag 3 was used as a dispersant–crude oil bag (15 g of Corexit 9527, 150 g of Shengli crude oil, and 3.7 MBq of n-(1-^{14}C)-hexadecane were added to achieve an initial concentration of about 1.5 $mg \cdot L^{-1}$ Corexit 9527, 15 $mg \cdot L^{-1}$ crude oil, and 370 $Bq \cdot L^{-1}$ radioactive tracer). Bag 4 was a duplicate of bag 3 without the added radiolabel, but it was not used to study the fate of crude oil.

Sampling

Bag 3 was sampled before the addition of oil and between the 1st h after treatment and day 14 at intervals of 1–3 d. Integrated seawater samples from 0–3 m depth were collected using a peristaltic pump. Sediment samples were removed through a plastic hose attached to the sediment collector at the bottom of the bags. Seawater for ^{14}C determination was collected using a Niskin sampler (Li, W., et al., this volume). Every 4–6 d, one of the three polyethylene ribbons (20 cm × 120 cm each), that were hung in the bag at the beginning of the experiment was collected. The ribbons were wrapped in aluminum foil and stored in a refrigerator for later determination of the amount of n-alkanes adhering to them (Wu, S., et al., this volume).

Parameters measured

Seawater temperature was measured at 1 m depth inside and outside the bags. Salinity was determined using a salinometer (HD-2 bridge model) calibrated with China Standard Seawater.

The dry weight of sediment was determined by weighing settled material collected on a 1.2-µm fibreglass filter (47-mm Whatman GF/C) that was predried at 60°C for 24 h. The filtered sample was dried at 450°C for 8 h and weighed with the filter. The dry weight of the sediment was determined by subtracting the filter weight from the total.

Particulate oil concentrations were determined by direct weighing of the extracted oil as suggested by Wong et al. (1984). On each sampling day, seawater and sediment samples were vacuum filtered through a 0.7-µm fibreglass filter (47-mm Whatman GF/F). The oil and associated particulates were treated with EtOH/KOH at 70°C for 1.5 h, to which an equal volume of hydrocarbon-free water was added for the last 0.5 h. The oil was recovered by consecutively extracting the filter paper contents and the digestion separately, first with CH_2Cl_2:EtOH (8:1) and then with redistilled CH_2Cl_2. The CH_2Cl_2 solution was washed with hydrocarbon-free water, dried over anhydrous Na_2SO_4, concentrated in a rotary evaporator, and diluted with pure CH_2Cl_2 to a standard volume. An aliquot was transferred to a weighing vessel, the solvent evaporated, and the residue weighed.

Filterable oil concentrations in the water column were determined gravimetrically (Wong et al. 1984). Filtrates of water samples that passed through a 0.7-µm filter (47-mm Whatman GF/F) were extracted with two portions of redistilled CH_2Cl_2 while shaking on a vibrator. The extracts were dried over anhydrous Na_2SO_4, concentrated, and made up to a standard volume. The extracted oil was determined as described above.

Alkanes on the polyethylene ribbons (20 cm × 120 cm) were extracted in redistilled CH_2Cl_2 by moving the ribbon up and down for 10 min. Oil adsorbed on the inner wall of the enclosure was measured gravimetrically after extraction into redistilled CH_2Cl_2. Extracts were dried over anhydrous Na_2SO_4 and then the solvent was evaporated from aliquots for gravimetric determination of oil.

Oil droplets associated with bacteria and diatoms were observed directly using a Zeiss Standard-18 microscope fitted with a phase-contrast condenser.

Results

Variations in temperature and salinity in bag 3 throughout the experiment are shown in Fig. 1. Water temperatures inside and outside the bag were very similar. The inside temperature at 1-m depth increased from 19.5°C at the beginning of the experiment to 25.9°C at the end of the experiment. The daily variation generally

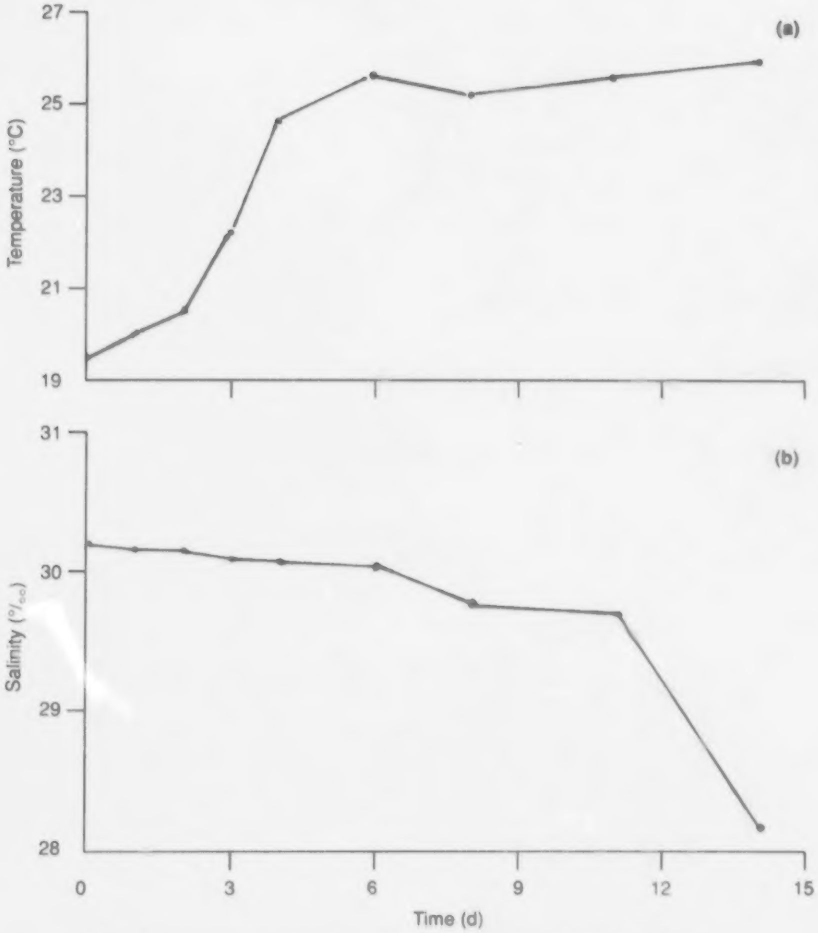

Fig. 1. Temperature (a) and salinity (b) variations at 1 m depth in bag 3.

fluctuated between 0.3 and 0.5°C, except from day 3 to day 6 when the seawater temperature went up rapidly and varied between 1.0 and 2.4°C. Salinity in bag 3 decreased gradually from 30.197‰ at the beginning of the experiment to 29.719‰ by day 11, decreasing more rapidly to 28.179‰ by the end of the experiment.

Figure 2 shows changes in concentrations of oil extracted from the water column, particulates, and settled materials as a function of time. The oil–dispersant mixture was injected at 0–3 m depth at the beginning of the experiment, giving an initial oil concentration of about 15 mg·L^{-1}. On day 1, the concentration of filterable oil in the seawater reached 2.6 mg·L^{-1}. From day 1 to day 4, it declined rapidly to 0.75 mg·L^{-1}, and stayed below 1.0 mg·L^{-1} until day 14. The oil recovered was about 12.1 g, or 8% of the total added (Tables 1 and 2). On day 1, the concentration of oil associated with particulates reached 11.0 mg·L^{-1} (Fig. 2). On day 2, it

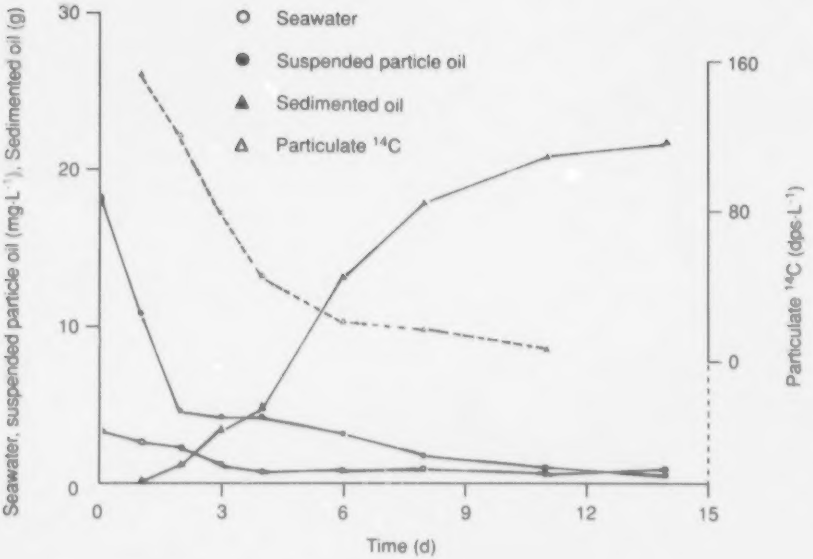

Fig. 2. Oil concentration in filtrate (<0.7 μm) seawater, associated with suspended particles (>0.7 μm), in the sediment (>1.2 μm), and in suspended particulate (>0.45 μm) ^{14}C in bag 3.

dropped rapidly to 4.65 mg·L^{-1}, then gradually decreased to 0.85 mg·L^{-1} between day 3 and day 11, remaining at about that level until the end of the experiment. About 8.5 g of the oil, or 6% of the total added, was recovered from the particles. Figure 2 also shows changes in the concentration of particulate oil in the settled materials. In contrast to filterable and particulate oil in the water column, the content of particulate oil in settled materials was almost negligible on the day 1, but accumulated to 22.1 g, or about 15% of the total added, by day 14.

During the experiment, about 20 cm of oil film surrounded the inner wall of bag 3 just above the waterline. The oil (tar and wax) adhering to the wall above and below the waterline was about 40.6 g, or about 27% of the total (Tables 1 and 2). The total amount of oil recovered from the water column, suspended particulates,

Table 1. Percentage of oil recovered from bag 3.

Day	Seawater	Particulates	Sediment	Wall	Total
1	21	69	0	—	—
2	20	40	1	—	—
3	11	37	2	—	—
4	7	37	3	—	—
6	8	28	8	—	—
8	8	15	12	—	—
11	6	7	14	—	—
14	8	6	15	27	56

Table 2. Quantity (g) of total oil and n-alkanes recovered from bag 3.

Medium	Total oil	n-alkanes[a] C11–C20	C21–C25	C26–C30
Particulates	8.5	0.05	0.02	0.08
Seawater	12.1	0.08	0.10	0.01
Sediment	22.1	0.30	0.33	0.50
Wall	40.6	0.92	3.21	2.06
Oil added	150	9.92	4.96	3.77

[a] Sources: MEEE Group (1986) and Wu et al. (this volume).

settled materials, and inner wall of the bag was about 83.3 g or 56% of the total added (Tables 1 and 2). The activity of ^{14}C in suspended particles (Li et al., this volume) is also shown in Fig. 2. The trend in radioactivity was very similar to variations in the concentration of particulate oil.

Figure 3 shows the settling rates of oil and particulates in bag 3. Maximums of 4.2 and 7.3 g·d^{-1} were reached for settled oil and settling materials, respectively, on day 6 after the oil/dispersant mixture was added. Thereafter, the settling rates declined rapidly with time.

Figure 4 shows the relationship between hydrocarbon concentration and numbers of oil-degrading bacteria. The number of bacteria increased quickly from 3.20 (log) cells·(100 mL)$^{-1}$ on day 2 to a peak value of 4.20 (log) cells·(100 mL)$^{-1}$ on day 8 (MEEE Group 1986), which coincides with the time when the hydrocarbon concentration in the water column was reduced to background levels.

Photographs taken through the phase-contrast microscope showed adherence between diatoms and oil droplet clusters, and the supposed oil-degrading bacteria growing around the oil droplets.

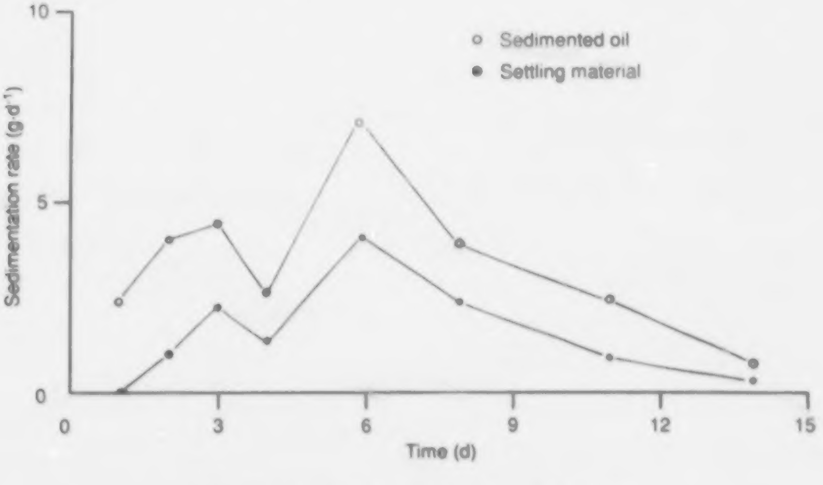

Fig. 3. Settling rate of oil and sediment in bag 3.

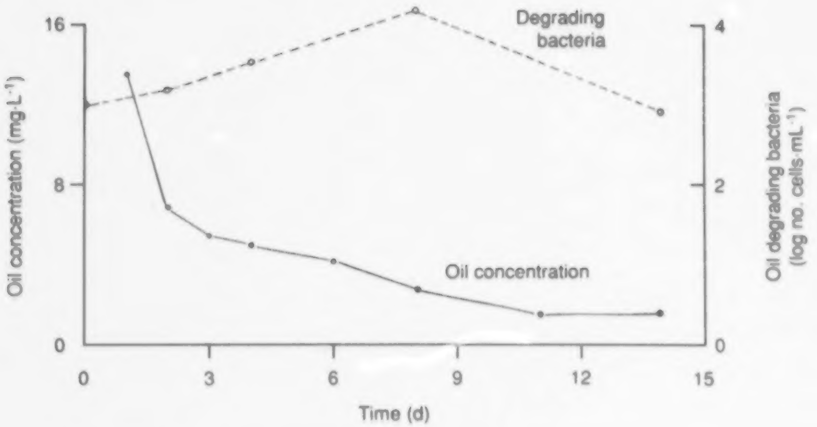

Fig. 4. Relationship between the number of oil-degrading bacteria and the oil concentration in bag 3.

Discussion

During the course of the 1986 Xiamen MEEE, the salinity of the seawater in the bags decreased about 2 ‰ from 30.197 to 28.179 ‰. Seawater might have seeped into the experimental bag through the sewn seams in the wall. Dahl et al. (1983) obtained a similar 2 ‰ reduction in salinity in their study of an enclosed ecosystem involving the addition of Ekofisk crude oil in the North Sea. They argued that the salinity reduction would not affect the experimental results. The oil–dispersant mixture, added to the upper 3 m of the water column, quickly distributed into a filterable phase and the particulate phase (nonfilterable fraction) with the aid of diffusion and convection processes. One day after adding the oil–dispersant mixture, about 21% of the oil was in the filtrate and 69% was associated with natural particles (Table 1). About 52% of the ^{14}C-labeled n-hexadecane was also associated with the particles (Li, W., et al., this volume).

The oil recovered from the water, particle, and sediment phases and the inner wall was 56% of the total amount added (Table 1). Although part (especially low molecular-weight hydrocarbons) of the 44% not recovered may have evaporated into the atmosphere through the air–sea interface, part was definitely biodegraded (Li, W., et al., this volume; Wu, W., et al., this volume). Thus, biological degradation in an enclosed system is an important process for oil removal.

Stimulated by the addition of oil, bacterial biomass in the water column increased rapidly from day 2 to a peak on day 8 (Fig. 4) and then decreased again to below the initial concentration by day 14. Oil-degrading bacteria were probably chemotaxic (Lin et al. 1986) and had a strong tendency to grow in association with oil droplets. The higher the number of bacteria associated with oil droplets, the better the degradative and other biological removal processes seemed to be. Thus, the time of maximum numbers of oil-degrading bacteria coincided with the time of minimum particulate and filterable oil concentrations in the water column.

Suspended particles, such as organic material, silt, and detritus, tend to adsorb hydrocarbons in different forms (Meyers and Quinn 1973; Bassin and Ichiye 1977; Lee 1977; Lee et al. 1982). These particles then settle to the bottom, which is an important process for removing oil from the water column. Results from the present study indicate that the settling rate was closely linked with phytoplankton productivity (Lin Yu et al., this volume).

In the control, nutrient concentrations were quite high at the beginning of the study. Diatoms reproduced rapidly, causing an increase in primary productivity. The settling rate reached its peak on day 2, then decreased gradually when the primary productivity was affected by nutrient depletion. In bag 3, however, diatom growth was suppressed by the added hydrocarbons. Specifically, centric diatoms decreased conspicuously during the first 3 d of this study, then rose again slowly, but never returned to their initial numbers, and primary productivity was also kept at a low level. Although pennate diatoms increased slowly, there was still no dominant species in bag 3 at the end of this study. Exhaustion of phosphate and nitrate during the first 4 d, and the stable concentration of silicate throughout the whole experimental period in bag 3 also reflected that the growth of diatoms was seriously suppressed. Thus, during the first few days, the settling rate remained low. As the number of pennate diatoms increased, the sedimentation rate showed a small peak on day 6, but it dropped again by day 8.

The settling rates of particulate oil and suspended were closely related (Fig. 3). The settling rates of both particulate oil and suspended particulate matter in bag 3 reached their maxima on day 6. This coincidence was caused by the association of oil-droplet aggregates with diatoms, which then settled to the bottom together. However, settling rates, which averaged 3.2 $g \cdot d^{-1}$ with a maximum of 7.3 $g \cdot d^{-1}$, were much lower than those obtained in the 1983 experiment, with an average of 8.0 $g \cdot d^{-1}$, with a maximum of 24.0 $g \cdot d^{-1}$.

There were several reasons for these differences. First, the initial and final concentrations of oil in this study were about 3–5 times higher than those used in the 1983 experiment. The higher concentrations seriously suppressed the growth of diatoms and zooplankton so that less organic matter was produced and settling rates were also reduced. Second, in the 1983 experiment, the enclosed water columns were especially undisturbed, whereas those in the present study were disturbed by strong local currents reaching a maximum of 1 $m \cdot s^{-1}$ during regular tidal action. In addition, several strong waves were encountered during the experimental period. The waves increased the level of disturbance, enhancing resuspension of settled material and thereby reducing settling rates. For instance, large storm-driven waves occurred on day 4, causing the sedimentation rate to drop suddenly to 2.7 $g \cdot d^{-1}$.

From day 2 of the study, the concentration of hydrocarbons in the enclosed water column decreased quickly with a rapid increase in settling rate, whereas the amount of hydrocarbons in the sediment increased constantly. After day 6, when the hydrocarbon concentration in the water column decreased and the settling rate slowed, the amount of hydrocarbons arriving at the sediment also decreased correspondingly. Thus, there was no obvious increase in the amount of hydrocarbons in the sediment during the latter part of the experiment.

When the enclosed water body was seriously disturbed, some settled materials were resuspended, which affected the settling process of hydrocarbons in the water column. This can be substantiated by the following facts. From day 3 onward, concentrations of filterable oil in the water remained almost constant (ca.

$1.0\ mg{\cdot}L^{-1}$). Between days 3 and 4, concentrations of particulate oil increased slightly rather than decreased, which may have been caused by resuspension of settled materials. As noted above, a storm during this period resulted in a marked decrease in sedimentation rates. During the same period, there was a slight dip in filterable-oil concentrations that may be attributable to the association of some previously filterable oil and oil–particulate aggregations with larger particles or to dilution by filterable oil of a lower concentration in water from below. The change in salinity with time indicated that the major infusion of outside water occurred much later in the experiment. Thus, the infusion of outside water was unlikely to have been involved in changes in oil concentrations observed during the enclosure in the first days of the experiment.

The present study showed that the removal of hydrocarbons from the enclosed water body may depend not only on biological degradation and particle sedimentation but also on the adherence of hydrocarbons to the plastic wall, which was an important process for removing oil from the water column. As the bag was rocked by tides and pounded by strong waves, the enclosed water body became seriously disturbed, which presumably increased the contact of particulate oil with the inner wall. In addition to the large amount of tar and wax adhering to the wall near the waterline, there was also a considerable amount of hydrocarbons adhering to the wall between the waterline and the conical end of the bag. By the end of the study, about 27% of the oil was recovered from the wall of the bag. This large percentage of oil recovered from the walls, compared with results from a previous study (Wong et al. 1984), could mainly be attributed to the disturbance and resuspension of settled materials, the larger surface-to-volume ratio in the bags, and the higher oil concentration used in this study compared with that used in the 1983 study.

At the beginning of the experiment, 150 g of crude oil was injected into bag 3. Of this oil, 83.3 g was recovered, or about 56% of the total added (Tables 1 and 2). Of this amount, 40.6 g, or about 27% of the total added, was recovered from the wall; 22.1 g, or about 15% of the total added, of particulate oil settled onto the sediment; and 12.1 and 8.5 g (8 and 6%) remained in suspension as dissolved or filterable fine particulate oil and nonfilterable particulate oil respectively. By direct measurements, 44% of the oil added was not recovered, of which part was degraded by chemical and biological processes and part was probably volatilized into the atmosphere.

The fate of crude oil added to experimental enclosures cannot be determined completely because not all existing forms and pathways of hydrocarbons are known. In a previous study, some floc formation was observed under a surface oil slick treated with the dispersant Corexit 9527 (Cretney et al. 1981). Observations by epifluorescence microscopy have identified the oil–organic particulate aggregations probably resulting from microbial growth. Some mesocosm studies found that 4–38%, or even up to 50%, of the hydrocarbons added could be found in oil associated with particulates or settling materials (Lee et al. 1978; Gearing et al. 1980). Observations by phase-contrast microscopy in this study also confirmed the clustering of oil droplets that may be attributable to bacterial growth and production of exopolymers.

Severe disturbance of the enclosed water body, which was not expected with the experimental design used, caused resuspension of settled material and slowed the rate of sedimentation. It affected the pathways and fate of hydrocarbons, and induced stress on the experimental ecosystem. However, it may be argued that the results obtained from this disturbed condition may be closer to those that would be obtained in the open ocean, even though the situation in the open ocean is more complicated. Further studies involving enclosed ecosystems should focus on biological processes in the sediment; physical disturbance by waves, causing resuspension of settled materials; and hydrocarbons in the surface microlayer.

In addition to studies on hydrocarbon reservoirs, the fate of aromatic hydrocarbons should also be considered as this complex of compounds in crude oil is fairly toxic to organisms. As such, it is suggested that future studies on the fate and pathways of different kinds of crude oil and their effects on ecosystems must be carried out to meet the needs of marine environmental protection and oil-pollution control.

Acknowledgment

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Bassin, N.J., Ichiye, T. 1977. Flocculation behaviour of suspended sediments and oil emulsions. Journal of Sedimentary Petrology, 47, 671–677.

Cretney, W.J., MacDonald, R.W., Wong, C.S., Green, D.R., Whitehouse, B., Geesey, G.G. 1981. Biodegradation of a chemically dispersed crude oil. *In* Proceeding of the 1981 Oil Spill Conference, 2–5 March 1981, Atlanta, GA, USA. American Petroleum Industry, Washington, DC, USA. Publication 4334, 37–43.

Dahl, E., Laake, M., Tjessem, K., Eberlein, K., Bohle, B. 1983. Effects of Ekofisk crude oil on an enclosed planktonic ecosystem. Marine Ecology Progress Series, 14, 81–91.

Davies, J.M., Baird, I.E., Massie, L.C., Hay, S.J., Ward, A.P. 1980. Some effects of oil-derived hydrocarbons on a pelagic food web from observations in an enclosed ecosystem and a consideration of their implications for monitoring. Rapport et Proces-Verbaux des Reunions, Conseil International pour l'Exploration de la Mer, 179, 201–211.

Gearing, P.J., Gearing, J.N., Pruell, R.J., Wade, T.L., Quinn, J.G. 1980. Partitioning of No. 2 fuel oil in controlled estuarine ecosystems: sediments and suspended particulate matter. Environmental Science and Technology, 14, 1 129–1 136.

Harrison, P.J., Cochlan, W.P., Acreman, J.C., Parsons, T.R., Thompson, P.A., Dovey, H.M. 1986. The effects of crude oil and Corexit 9527 on marine phytoplankton in an experimental enclosure. Marine Environment Research, 18, 93–109.

Lacaze, J.C. 1974. Ecotoxicology of crude oils and the use of experimental marine ecosystems. Marine Pollution Bulletin, 5, 153–156.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parsons, T.R., Lalli, C.M., Wu, J. 1985. Microbial responses to crude oil and Corexit 9527: SEAFLUXES enclosure study. Microbial Ecology, 11, 337–351.

Lee, R.F. 1977. Fate of petroleum components in estuarine waters of the southeastern United States. *In* Proceedings of the 1977 Oil Spill Conference, 8–10 March 1977, New Orleans, LA, USA. American Petroleum Institute, Washington, DC, USA. 611–616.

Lee, R.F., Takahashi, M., Beers, J.R., Thomas, W.H., Seibert, D.L.R., Koeller, P., Green, D.R. 1977. Controlled ecosystems: their use in the study of the effects of petroleum hydrocarbons on plankton. Vernberg, F.J., Calabrese, A., Thurberg, F.P., Vernberg, W.B., ed., *In* Physiological responses of marine biota to pollutants. Academic Press, London, UK. 323–342.

Lee, R.F., Gardner, W.S., Anderson, J.W., Blaylock, J.W., Barwell-Clarke, J. 1978. Fate of polycyclic aromatic hydrocarbons in controlled ecosystem enclosures. Environmental Science and Technology, 12, 832–838.

Lee, R.F., Hinga, K., Almquist, G. 1982. Fate of radiolabelled polycyclic aromatic hydrocarbons and pentachlorophenol in enclosed marine ecosystems. *In* Grice, G.D., Reeve, M.R. ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 123–135.

Lin, Y., Yao, R., Lian, Z. 1986. The effects of crude oil and dispersant (Corexit-9527) on the early microfouling organisms in MEEE. Paper presented during the Regional Seminar on MICE III Brackish Water Ecosystems: with emphasis on conservation and management, held in Ranong Province, Thailand, 9–12 December 1986.

MEEE Group. 1986. Addition of hydrocarbon and dispersant Corexit 9527 into marine enclosed experimental ecosystems. *In* Data report of the Marine Ecosystem Enclosure Experiment Group. Third Institute of Oceanography, Xiamen, People's Republic of China. Unpublished.

Meyers, P.A., Quinn, J.G. 1973. Association of hydrocarbons and mineral particles in saline solution. Nature, 244, 23–24.

Parsons, T.R., Harrison, P.J., Acreman, J.C., Dovey, H.M., Thompson, P.A., Lalli, C.M., Lee, K., Li, G., Chen, X. 1984a. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 2: Biological effects. Marine Environment Research, 13, 265–275.

Wong, C.S., Whitney, F.A., Cretney, W.J., Lee, K., McLaughlin, F., Wu, J., Fu, T., Zhuang, D. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 1: Fate of chemically dispersed crude oil. Marine Environmental Research, 13, 247–263.

Fate of Low-Volatility Alkanes from Chemically Dispersed Crude Oil in a Marine Ecosystem Enclosure

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The composition of n-alkanes (nC11–nC36) in each phase of the experimental ecosystem treated with chemically dispersed crude oil was determined by capillary gas chromatography. Low-volatility n-alkanes quickly associated with suspended particles after Shengli crude oil with Corexit 9527 (10:1 w/w) was homogeneously distributed in the upper 3 m of the enclosed water column at an oil concentration of about 15 mg·L^{-1} of water. Thereafter, most of the shorter chain n-alkanes (nC11–nC20) were degraded by microbes and the longer chain n-alkanes (nC21–nC31) adhered to the wall of the experimental bags. In addition, some of the n-alkanes (nC15–nC32) also settled on the sediment. Results showed that few of the n-alkanes remained in the filterable–dissolved phase and suspended particulate phase (about 1% each). About 40% of the n-alkanes added was recovered from sediment and the inner wall and about 58% was removed by microbial and other processes.

In the marine environment, crude oil can be removed quickly from the seawater surface by using chemical dispersants. Chemical dispersion is regarded as an effective method for cleaning-up oil, especially when large oil spills threaten a coastline. The behaviour of chemically dispersed oil and the biological effects of dispersants and dispersed oil are topics worthy of scientific investigation.

In 1983, a marine ecosystem enclosure experiment (MEEE) was conducted in Saanich Inlet, BC, Canada, to study the fate of crude oil and dispersant (Wong et al. 1984). In 1986, a similar experiment was carried out in Xiamen, People's Republic of China. Corexit 9527 was used in both experiments, although in the Xiamen experiment crude oil from the Shengli oil field in China was used instead of the Prudhoe Bay crude oil used in the Saanich Inlet experiment. The enclosed water body in the Saanich Inlet experiment was 65 m^3; that in the Xiamen experiment was about 13 m^3. The marine ecosystem in the two experimental areas differed substantially, but results showed remarkable similarities. Because the former experiment did not look at the behaviour of alkanes in detail, even though they are prominent constituents of oil pollution in marine environments, the Xiamen experiment included the fate of alkanes as a major component of the study.

Methods

Field methods

The experimental design and field sampling methods are described by Zhuang et al. (this volume). Sample extraction is also described by Zhuang et al. (this volume).

After gravimetric determination, the extracts were analyzed for oil composition and concentration using a calibrated capillary gas chromatograph (Varian Model 3760). The calibration standard consisted of a mixture of pristane, phytane, and all of the n-alkanes from nC11 to nC36 except nC33. The injection standard used was pristane-d_{40}. The chromatographic column used was a J & W fused silica capillary column (30 m × 0.321 mm) coated with SE-54. The concentrations in the extracts of the major alkane components in the C11–C36 range that were resolved by gas chromatography were determined.

Results

Analysis of the composition of the crude oil from Shengli oil field revealed that n-alkanes from nC11–nC36, plus pristane and phytane, but excluding nC33 because it had no standard, accounted for about 14.7% of the crude oil. Of the 150 g of crude oil added to the enclosure, measured alkanes accounted for about 22 g.

Figure 1 shows the changes in the total amount of alkanes in various phases in

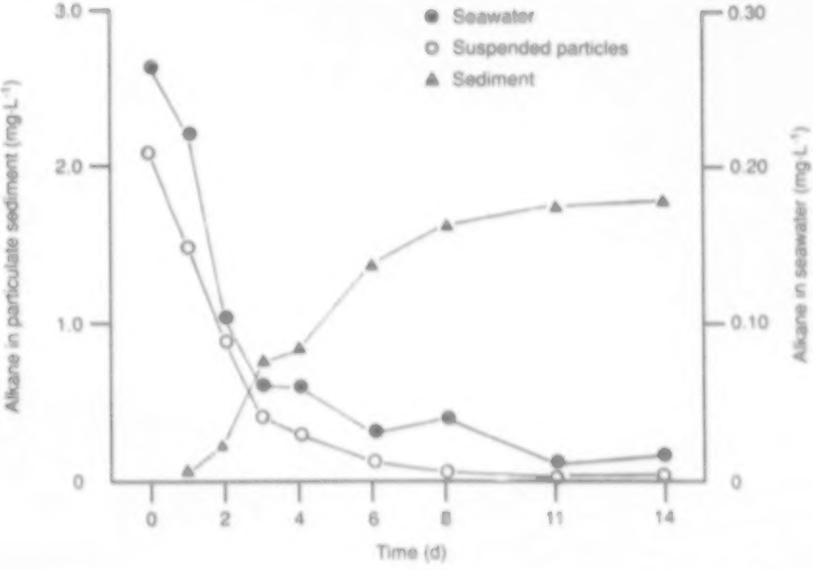

Fig. 1. Alkane concentration in filtrate seawater, absorbed onto suspended particles, and in the sediment.

Table 1. Percentage of added alkanes recovered in bag 3.

Medium	Day								
	0[a]	1	2	3	4	6	8	11	14
Seawater	12	10	6	4	4	2	2	0.5	1
Particle	86	86	52	23	17	7	3	1	1
Sediment	—	0	1	3	4	6	7	8	8
Wall	—	—	—	—	—	48[b]	—	—	32[b]
Total	—	—	—	—	—	63	—	—	42

[a] One hour after addition of the oil–dispersant mixture.

[b] Determined from suspended ribbons of bag wall material.

bag 3 during the experiment. As the oil–dispersant mixture was added to the enclosure, chromatographically resolved alkanes in the crude oil dispersed very quickly and the contents of alkanes in water in filterable or nonfilterable (0.7-μm Whatman GF/F) phases were 0.26 and 2.1 $\mu g \cdot L^{-1}$, respectively.

Although the crude oil was initially dispersed in the upper 3 m of the water column, it quickly mixed into the whole water column within 1 d. At this time, nearly all of the resolved alkanes (96%) remained in suspension in the water (Table 1). The concentration of these alkanes decreased rapidly thereafter. By day 6, only 9% of the alkanes remained in the water column; by the end of the experiment, only 1% remained. On day 1, there was only a small quantity of alkanes in the sediment. From day 2 to day 8, more alkanes were carried down to the sediment. The alkanes recovered from the sediment accounted for about 8% of the total alkanes added. At the end of the experiment, about 32% of the alkanes added were estimated to be in oil coated on the enclosure wall (Table 1). Gravimetric determinations also revealed that about 27% of the crude oil added adhered to the enclosure wall (Zhuang et al., this volume). These results indicated that adhesion to the inner wall of the enclosure was quite important as a removal process.

Figure 2 shows the concentration and composition of the major low-volatility alkanes in the Shengli crude oil as determined by gas chromatography. The major components that were chromatographically resolved were mainly in the nC11–

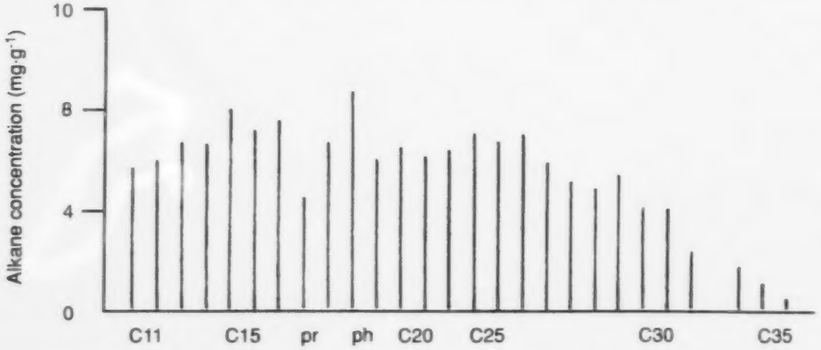

Fig. 2. Concentration and composition of alkanes in crude oil initially added to bag 3.

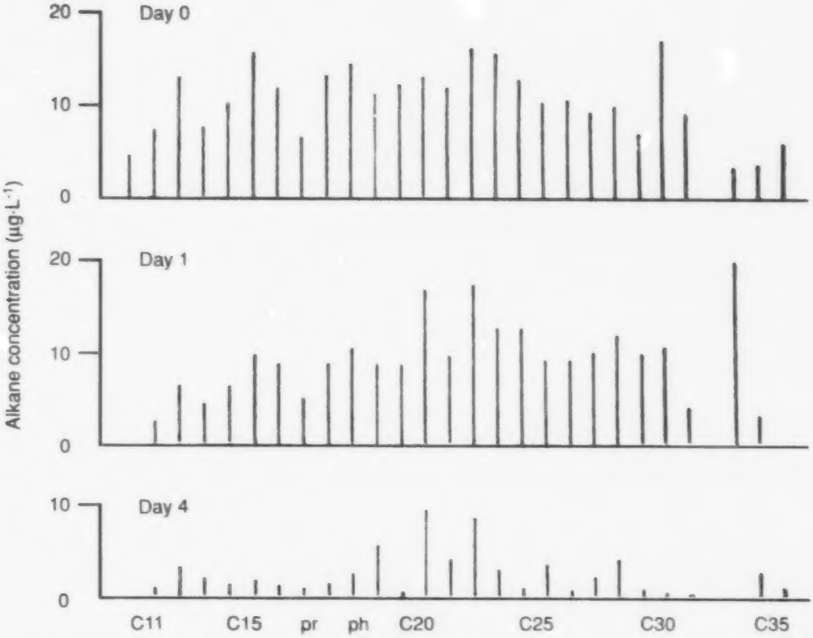

Fig. 3. Concentration and composition of alkanes in filtrate from bag 3 on days 0, 1, and 4.

nC29 range, which accounted for 91% of the total C11–C36 alkanes. Figures 3–6 show the changes in alkane concentrations in filterable and nonfilterable aqueous phases, sediment, and oil adhering to the wall of the enclosure respectively.

One hour after the addition of crude oil, the alkane components found in the filterable portion of the seawater were mainly in the C15–C32 range and the content of shorter chain C11–C14 alkanes (11%) relative to the total n-alkanes was lower than that in the crude oil (17%). This result could reflect volatility losses from the enclosure to the atmosphere or to vacuum during filtration. The relative content of C11–C14 alkanes (20%) in particles, however, was slightly higher than that in the crude oil.

Because Corexit 9527 is known to generate submicrometre oil droplets (Shaw and Reidy 1979), these results may be explained given that, during and immediately after the addition of oil to the water, the shorter chain C11–C14 alkanes dissolved and diffusively partitioned more quickly to nonfilterable, micrometre-sized and larger particles than the generated submicrometre oil droplets, which became mechanically associated with the same larger particles. At the same time, the limited solubility of the higher molecular weight n-alkanes would retard their chemical partitioning by dissolution, solution-phase transport, and adsorption or absorption by particles.

After day 1, the composition of the alkanes began to change. The shorter chain n-alkanes in the seawater and on particles decreased more rapidly than the longer chain n-alkanes. The composition of n-alkanes in sediments was always dominated

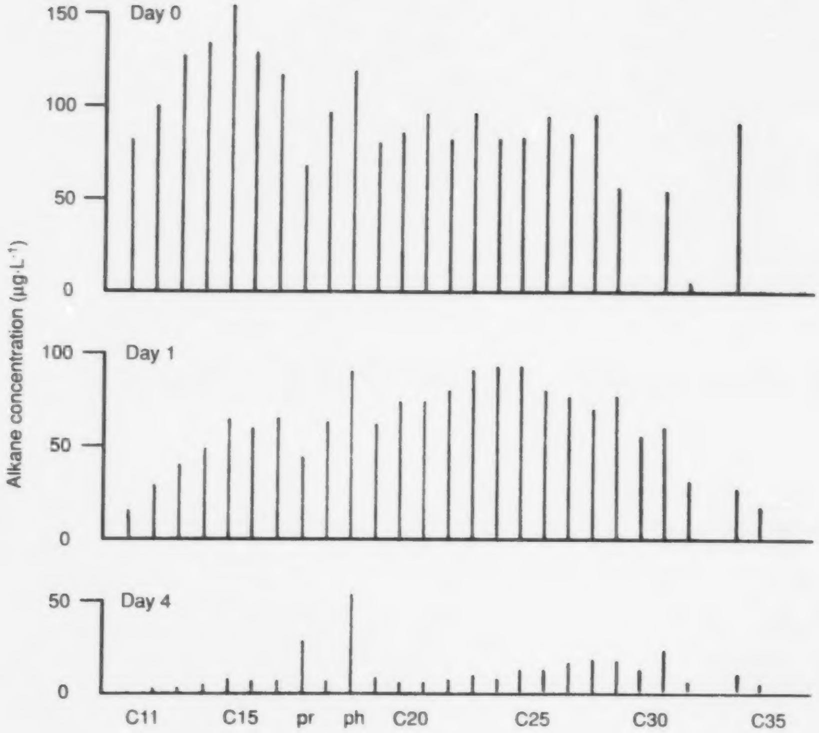

Fig. 4. Concentration and composition of alkanes from particles from bag 3 on days 0, 1, and 4.

by longer chain alkanes, with a maximum at nC31; whereas the material that adhered to the wall showed a maximum at nC25. The temporal change in alkane composition in the various phases showed that the disappearance of shorter chain C11–C20 n-alkanes, was very significant.

The settling rate of total alkanes reached its maximum 3 d after the addition of crude oil. However, the settling rate of suspended particulate matter and total oil peaked on day 6 (Fig. 7), by which time the content of n-alkanes had decreased through biodegradation. As the content of particulate n-alkanes was very low by the day 6, the amount of longer chain alkanes carried down onto the sediment was not large, even though the amount of particles settling to the bottom was fairly substantial. The sedimentation rate for branched chain pristane and phytane, which are resistant to biodegradation, also peaked on the day 6.

Discussion

After crude oil was added to the enclosed water column, the alkanes distributed quickly between filterable and nonfilterable phases in the water column. Most of

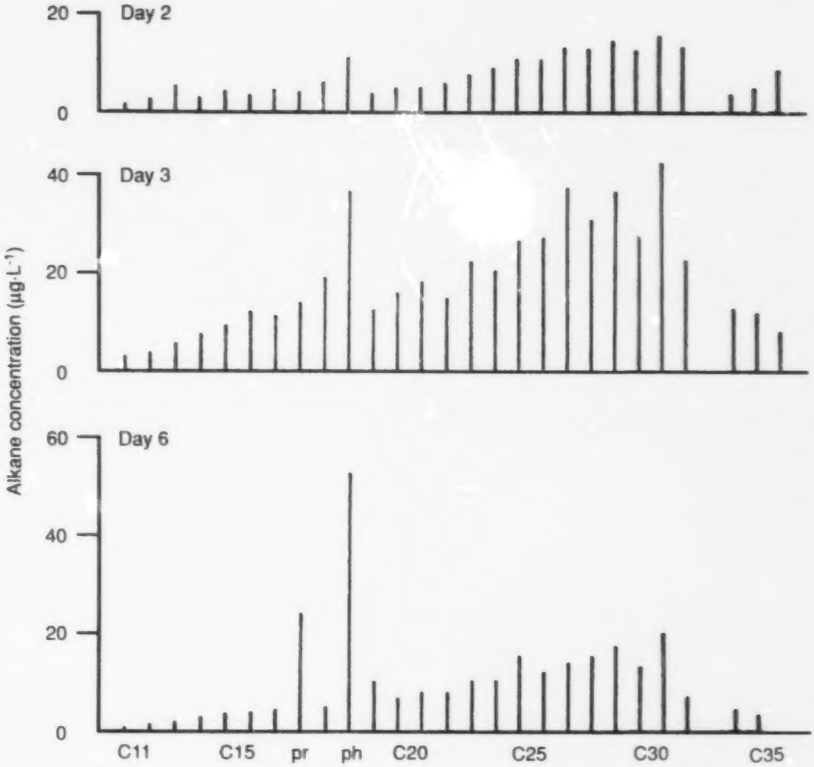

Fig. 5. Concentration and composition of alkanes from sediment on bag 3 on days 2, 3, and 6.

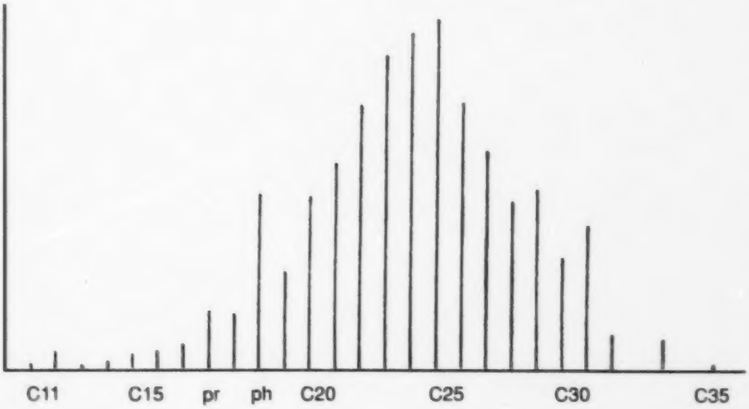

Fig. 6. Relative concentration of alkanes on the wall of bag 3 at the end of the experiment.

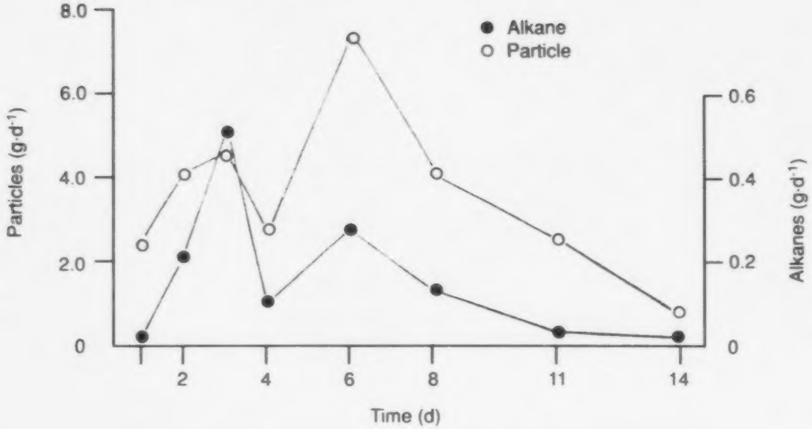

Fig. 7. Variation in the sedimentation rate of alkanes and particles.

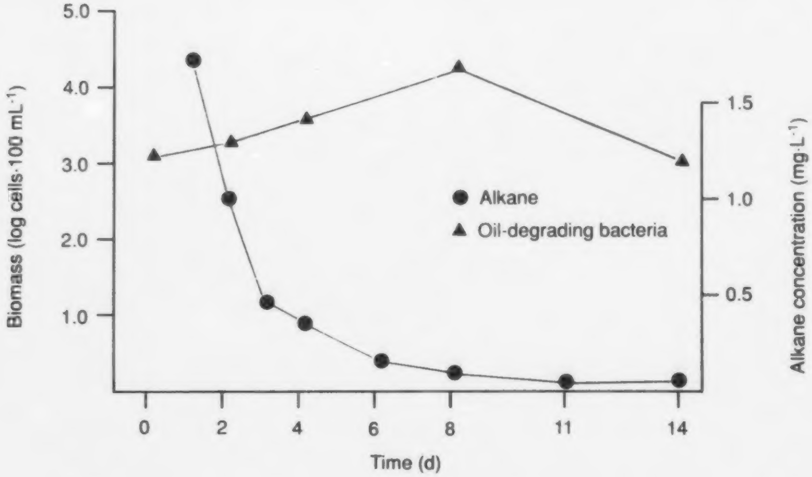

Fig. 8. Relationship between the concentration of alkanes and the biomass of oil-degrading bacteria in the water column in bag 3.

the alkanes existed in the latter phase because of the heavy association of alkanes with suspended particles. One hour after addition, the oil was, for the most part, homogeneously dispersed within the 0–3 m layer. One day later, fairly complete mixing of the enclosed water occurred because of tidal and wave action. As a result, the dispersed oil was distributed homogeneously throughout the whole water column. Consequently, the maximum concentration of alkanes in the water column on day 1 was lower than that determined 1 h after the addition of crude oil. However, most of the alkanes (96%) in the crude oil added remained in the water column after 1 d.

Alkanes in the water column decreased exponentially after day 1, reaching their background values by about day 8. The decrease was attributed not only to biodegradation, but also to adhesion to the bag walls. With the decrease in alkane concentration, the biomass of oil-degrading bacteria increased exponentially (Fig. 8). The biomass of oil-degrading bacteria began to decrease when the alkane concentration approached background concentrations.

The decrease in nC17:pristane and nC18:phytane ratios also reflected the effects of oil-degrading bacteria on the oil hydrocarbon (Blumer et al. 1973). The ratios of nC17:pristane and nC18:phytane in the filterable seawater component decreased from 1.78:1 to 0.44:1 and from 0.93:1 to 0.40:1, respectively. The initial concentration of alkanes on nonfilterable suspended particles was very high and ratios of nC17:pristane and nC18:phytane decreased (Fig. 9) more conspicuously from 1.74:1 to 0.09:1 and from 0.79:1 to 0.05:1, respectively, indicating more effective degradation of the nonfilterable particulate oil by bacteria. The two ratios tended to be constant after day 6 (Fig. 9), indicating that degradation by bacteria occurred mainly during the first 6 d, especially from day 1 to day 4 when the change in ratios was very rapid. This finding was supported by changes in the concentration of alkanes in the water column (Fig. 8).

Cretney et al. (1981) also studied biodegradation of chemically dispersed oil in two enclosed ecosystems. Their experiment differed from the present study in that oil was added at the surface to a larger volume of water (65 m^3). In the present experiment, oil was added homogeneously to the 0–3 m water layer of an enclosure with a volume of only 13 m^3. Thus, the initial distribution of oil in the water

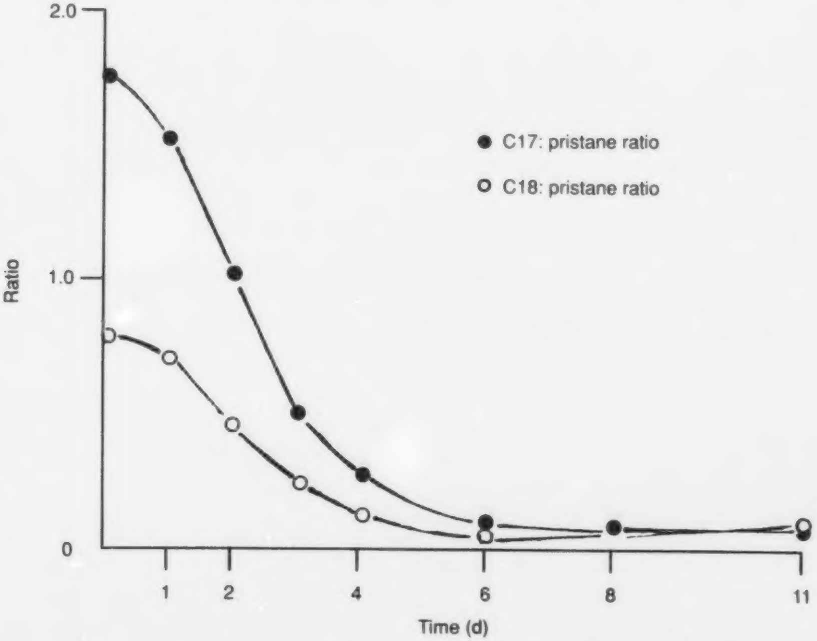

Fig. 9. Change in nC17:pristane and nC18:phytane ratios in suspended particles in bag 3.

columns was different. Also, the period for bacterial degradation in the present study was shorter than that in the study conducted by Cretney et al. (1981). Furthermore, the Xiamen experiment was conducted in seawater at temperatures ranging from 19.5 to 25.9°C. The high temperature would be expected to accelerate bacterial growth and oil degradation.

Major straight- and branched-chain alkanes accounted for about 15% of the original crude oil. After the crude oil was added, the ratios of these alkanes to extractable oil in the different phases of the dispersed crude oil changed immediately (Fig. 10). The percentage of these alkanes in filterable oil decreased initially, increased again on day 4, and then decreased again. The percentage of alkanes in oil adsorbed on particles increased initially, but then decreased rapidly. The percentage decrease in the particle oil was, evidently, a result of biodegradation. During the first 2 d, the percentage increase in alkanes in the particle oil that was mirrored by a percentage decrease in the filterable oil may have been due to a partitioning phenomenon. The similarity in the percentage of alkanes in filterable, nonfilterable, and sedimentary oil on day 4 may have been coincidental, but more likely represented sediment resuspension and homogenization processes affecting the particle-size distribution as a result of a storm that occurred on that day.

There were large changes in oil composition as different components of chromatographically resolved alkanes in the enclosed water body were lost as a result of biodegradation processes, adsorption on the wall, and sedimentation. During the experiment, there were some evaporative losses of the more volatile oil constituents, especially the short chain C11–C14 alkanes. However, the chemically

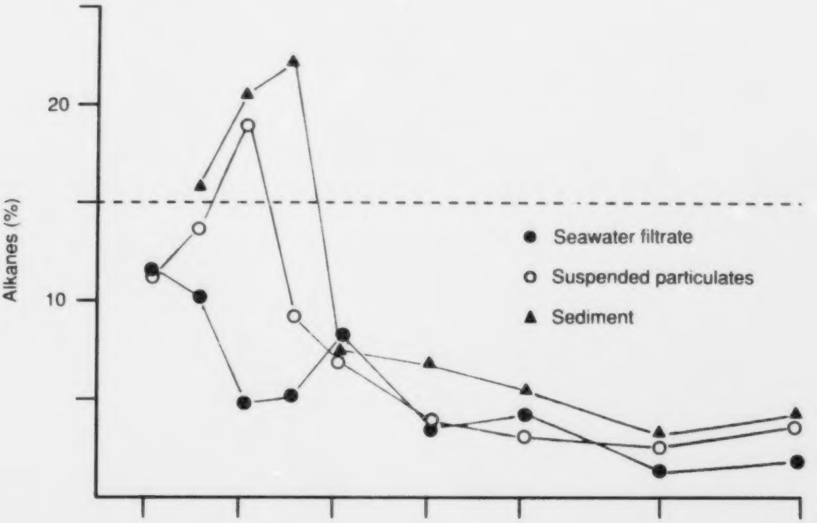

Fig. 10. Variation in the percentage of alkanes in extractable oil in different phases in bag 3: seawater filtrate, suspended particulates, and sediment. Broken horizontal line is percentage of alkanes in crude oil (14.7%).

Table 2. Percentage of added alkanes in three molecular weight ranges recovered from various phases in bag 3.

	Wall	Sediment	Seawater	Particulate
C11–C20	9.3	3.0	0.8	0.5
C21–C25	64.7	6.7	2.0	0.4
C26–C30	54.6	13.3	0.3	2.1

dispersed oil was distributed homogeneously throughout the whole water column and most of it was adsorbed on or aggregated with suspended particles, thus evaporative losses were not serious except for the case where alkanes became coated to the enclosure wall at the air–water interface. Based on analytical results of recovered alkanes, most of the C11–C20 alkanes (86%) were removed from the water column. About 65% of the C21–C25 component and 55% of the C26–C30 component adhered to the bag wall (Table 2). As the number of carbons in an alkane increases, results indicate that the degradation rate by bacteria decreases (Ni et al. 1984).

This experiment, like previous marine ecosystem enclosure studies, has shown that the marine microbial community can respond in a timely manner to remove the n-alkane component from a dispersed oil spill. Further studies, perhaps of much longer duration, should be carried out to investigate the fate of hydrocarbons other than n-alkanes and the mechanism of biodegradation.

Acknowledgments

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Blumer, M., Ehrhardt, M., Jones, J.H. 1973. The environmental fate of standard crude oil. Deep-Sea Research, 2, 239–259.

Cretney, W.J., Macdonald, R.W., Wong, C.S., Green, D.R., Whitehouse, B., Geesey, G.G. 1981. Biodegradation of a chemically dispersed crude oil. *In* Proceedings of the 1981 Oil Spill Conference, 2–5 March 1981, Atlanta, GA, USA. American Petroleum Institute, Washington, DC, USA. Publication 4334, 37–43.

Ni, C., Zhon, Z., Cai, Z., Zeng, H., Li, Z., Xie, J., Zhang, N. 1984. A study of microbial degradation of oil pollutants in marine environments — 3: Degradation of petroleum hydrocarbons by marine microbes. Acta Oceanologica Sinica, 6, 497–504. [In Chinese.]

Shaw, D.G., Reidy, S.K. 1979. Chemical and size fractionation of aqueous petroleum dispersions. Environmental Science and Technology, 13, 1259–1263.

Wong, C.S., Whitney, F.A., Cretney, W.J., Lee, K., McLaughlin, F., Wu, J., Fu T., Zhuang, D. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 1: Fate of chemically dispersed crude oil. Marine Environmental Research, 13, 247–263.

Use of n-(1-^{14}C) Hexadecane to Study the Fate of Dispersed Crude Oil in Marine Enclosed Ecosystems

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The fate of Corexit-dispersed crude oil in an enclosed experimental ecosystem in Xiamen Bay was studied using n-(1-^{14}C) hexadecane as a tracer. The removal of crude oil was a two-step process. The first step, from day 1 to day 7, was a fast process, with a removal rate of $0.37\%\cdot d^{-1}$ characterized by nutrient limitation. The removal half-life was 2 d. The time required for forming organic floc was estimated to be 7 d, a 4-d delay from the bacterial bloom. The second step, from day 7 to day 15, was a slow process with a removal rate of $0.17\%\cdot d^{-1}$. The removal half-life was 4 d. Compared with the first step, dominated by heterotrophic bacteria, the mechanism of oil removal during the second step was much more complicated, probably because of changes in the environmental conditions of the seawater.

The study indicated that residual ^{14}C of suspended particulate matter, sediment, and dissolved organic carbon amounted to 3.4, 12.4, and 1% of the total tracer added respectively. The release of $^{14}CO_2$ was 25% of the added tracer. Considering its escape to the atmosphere and assimilation by phytoplankton, this value should be much higher. The experimental ecosystem, when contaminated with a limit of 15 ppm crude oil with a dispersant, was able to remove the oil by natural processes within 2 weeks.

Recently, studies of transfer processes and the fate of crude oil in the ocean have received considerable attention (Gearing et al. 1979; Lee 1980). In cleaning up Corexit-dispersed oil, biodegradation appears to be more important than abiotic processes (Cretney et al. 1981; Lee et al. 1985). However, some questions remain to be answered. For example, how to monitor the effects of biological processes on the transfer of crude oil? How to estimate the rate of oil removal? How much is primary productivity suppressed by oil pollution? What is the capability of a marine ecosystem in terms of the natural removal of crude oil? The use of radioisotopic techniques allows scientists to trace the pathways of crude oil by determining degradation products in the experimental ecosystem.

This study forms part of the marine ecosystem enclosed experiments (MEEE) sponsored jointly by Canada and the People's Republic of China.

Methods

Sampling

The experimental site was located at the eastern end of Xiamen Bay where the local current reaches 3 $km \cdot h^{-1}$. Four double-layered polyethylene bags (2 m in diameter × 6 m long) were filled with seawater. On 23 May 1986, one of the enclosures was injected with 150 g of Shengli crude oil (about 15 $mg \cdot L^{-1}$), 15 g of Corexit 9527 (about 1.5 $mg \cdot L^{-1}$) and 3.7 MBq of n-(1-^{14}C) hexadecane (about 370 $Bq \cdot L^{-1}$) in the upper 3 m of seawater using a sprayer. Seawater was sampled using a 2.5-L Niskin sampler at 0, 1.5, 2.5, and 4 m depth for tracer analyses.

Analyses

^{14}C in suspended particulate matter

Suspended particulate matter (SPM) in each 500 mL of seawater sample was filtered onto either a 47-mm Millipore filter (0.45 μm) or a 47-mm Nuclepore filter (3 μm or 8 μm) at a suction pressure of about 34 kPa. The filter and its content were then placed in a vial containing 10 mL of Aquasol. All ^{14}C samples were counted in a liquid scintillation counter (Packard, Tri-Carb 4640).

^{14}C in sediment

A 25-mL subsample of the sediment collected was mixed thoroughly. From this, a 1-mL aliquot was withdrawn and placed into a scintillation vial containing 10 mL of Aquasol. It was then counted as described above.

$^{14}CO_2$ measurement

A 500-mL gas-washer bottle with stopper was filled with seawater from the Niskin sampler. The bottle was connected with the air-circulating loop shown (Fig. 1). Before turning on the air pump, the sample was acidified with 1 mL of concentrated H_2SO_4. Air was bubbled through the sample solution to purge CO_2 gas into a scintillation vial containing a 5-mL solution of $SrCl_2$ in NH_4OH (25% w/w). The CO_2 was absorbed over 15 min to form a precipitate of $SrCO_3$. A pinch clamp was used to control the bubbling rate to avoid "boiling" the sample. The scintillation vial was heated slowly to remove the NH_4OH until 2 mL of solution remained. This was mixed with 10 mL of Aquasol to form a homogeneous gel. The production of $^{14}CO_2$ was computed using the equation

$$[1] \quad {}^{14}CO_2\ (dpm \cdot L^{-1}) = 2(n_s - n_b)/(E_1 E_2)$$

where n_s is the counting rate of the sample (cpm), n_b is the counting rate of the blank (cpm), E_1 is the counting efficiency corrected for quenching, and E_2 is the chemical recovery efficiency. (Note: dpm = disintegrations per minute = 60 Bq.)

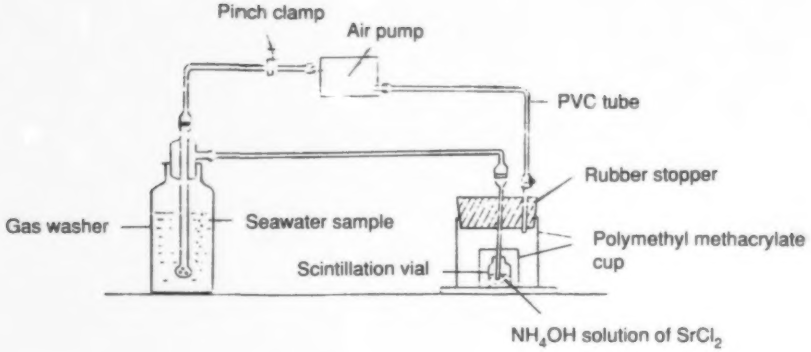
Pinch clamp
Air pump
PVC tube
Rubber stopper
Gas washer
Seawater sample
Polymethyl methacrylate cup
Scintillation vial
NH$_4$OH solution of SrCl$_2$

Fig. 1. Closed air-circulating loop.

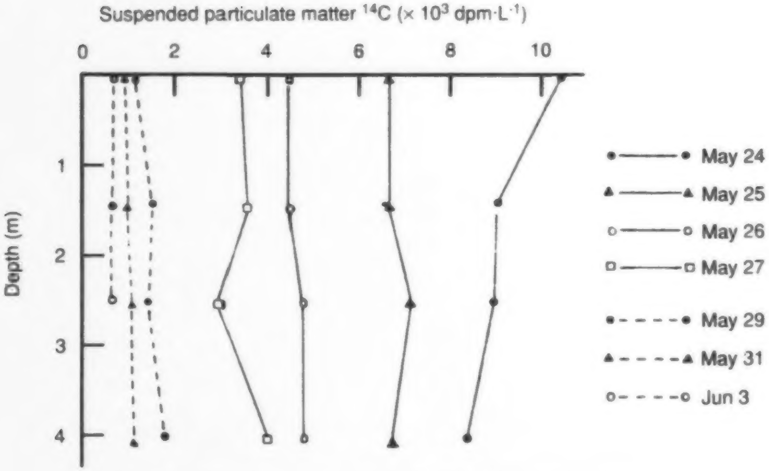

Fig. 2. Changes in suspended particulate ^{14}C in the water column.

^{14}C in dissolved organic carbon

The filtrate collected from the procedure followed to determine the ^{14}C in SPM using a 0.45-μm filter was extracted twice by shaking the sample with CH_2Cl_2 in a 1 000-mL separatory funnel. Volumes of CH_2Cl_2 used were 20 and 10 mL, respectively, with each extraction lasting 5 min. Both extracts were combined into a 50-mL graduated test-tube. The CH_2Cl_2 was evaporated at room temperature to a final volume of about 3-mL by bubbling nitrogen gas through the extract. For counting, 2-mL of the extract was used with 10-mL of Aquasol.

Bacterial productivity

The productivity of bacterioplankton was estimated using the method described by Fuhrman and Azam (1982) and Parsons et al. (1984). In this method, the amount

Table 1. Distribution of suspended particulate matter ^{14}C (suspended, 0.45-μm filter retained, dpm·L^{-1}) in the water column.

		Depth (m)				Integrated amount
Day	Date	0	1.5	2.5	4.0	(× 10^6 dpm)
1	23 May	23 047	—	—	—	—
2	24 May	10 207	9 004	8 939	8 314	114.5
3	25 May	6 702	6 634	7 082	6 776	85.4
4	26 May	4 391	4 455	4 700	4 695	57.3
5	27 May	3 391	3 520	2 775	3 902	42.7
7	29 May	1 080	1 510	1 359	1 653	17.6
9	31 May	884	902	1 135	1 052	12.5
12	3 June	613	601	582	—	7.5

of (methyl-^{3}H)-thymidine incorporated was converted to the total number of bacteria for determining heterotrophic production.

Results and discussion

Distribution of ^{14}C in the water column

The vertical distribution of SPM ^{14}C in the enclosure is shown in Fig. 2. On day 2, the average level of SPM ^{14}C was 9 100 dpm·L^{-1}. It was higher in the surface but lower in the deeper layer. The concentration decreased rapidly to 4 560 dpm·L^{-1} on day 4 and to a low of 600 dpm·L^{-1} on day 12. At the end of the experiment, the integrated amount of SPM ^{14}C remaining in the water column was 7.5 × 10^6 dpm, which was only 3.4% of the total tracer added (Table 1). The distribution curve shifted in a well-defined front, indicating a well-mixed condition in the enclosure.

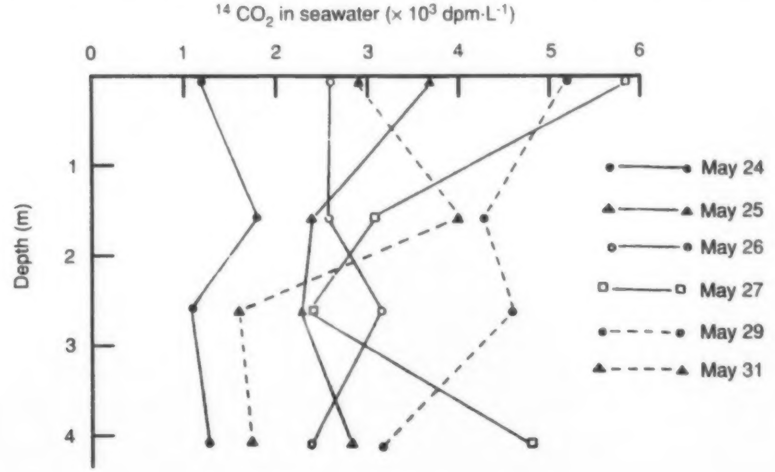

Fig. 3. Changes in ^{14}C in the water column.

The variability of $^{14}CO_2$ production in seawater is shown in Fig. 3. From day 2 to day 7, the integrated amount of $^{14}CO_2$ increased, reaching a peak of 54.53 × 10^6 dpm on day 7, which was 24.6% of the total tracer added (Table 2). After day 7, production of $^{14}CO_2$ decreased and spread evenly throughout the water column in the same way as SPM.

The amount of dissolved organic carbon (DOC) was very low (Table 3), mostly less than 1% of the total tracer. Even though the maximum integrated DOC was 1.56 × 10^6 dpm on day 5, it represented only 3.5% of the SPM plus DOC in the water. As the sediment samples were collected every day, the values of ^{14}C in the sediment were the sums of all the daily counts. Counting results of sediment ^{14}C (Table 4) showed a maximum of 10.84 × 10^6 dpm on day 7. A total of 27.6 × 10^6 dpm of sediment ^{14}C was attained, which was 12.4% of the total tracer added. Thus, 10% of the labeled hexadecane had sunk to the bottom.

Estimation of the crude oil removal rate

The actual production of $^{14}CO_2$ might be much higher than the concentration detected as some CO_2 probably escaped to the atmosphere or was assimilated by

Table 2. Released $^{14}CO_2$ (dpm·L^{-1}) and bacterial productivity.

		Depth (m)					
Day	Date	0	1.5	2.5	4.0	Integrated amount (× 10^6 dpm)	Bacterial productivity (× 10^6 cells·L^{-1}·h^{-1})
1	23 May	—	—	—	—	—	64
2	29 May	1 162	1 771	1 076	1 282	16.65	318
3	25 May	3 693	2 412	2 296	2 775	35.11	373
4	26 May	2 595	2 585	3 178	2 416	33.85	334
5	27 May	5 854	3 065	2 336	4 795	50.42	309
7	29 May	5 888	4 323	4 623	3 223	54.53	213
9	31 May	2 939	3 980	1 638	1 756	32.40	321
12	3 June	1 721	1 769	6 994	—	—	224
15	6 June	—	—	—	—	—	142

Table 3. Dissolved organic ^{14}C (dpm·L^{-1}) in the water column.

		Depth (m)						
Day	Date	0	1.5	2.5	4.0	Integrated amount (× 10^6 dmp)	% of total tracer	% of SPM + DOC
2	23 May	164	—	—	—	—	—	—
2	24 May	27	43	16	0	0.27	0.1	0.2
3	25 May	4	0	31	20	0.17	0.1	0.2
4	26 May	35	—	31	35	0.41	0.2	0.7
5	27 May	92	43	359	4	1.56	0.7	3.5
7	29 May	12	16	4	0	0.10	0.04	0.6
9	31 May	35	50	0	69	0.48	0.2	3.7

Note: SPM, suspended particulate matter; DOC, dissolved organic carbon.

Table 4. Sediment ^{14}C and its integrated amounts.

Day	Date	Sediment ^{14}C ($\times 10^6$ dpm)	Integrated amount ($\times 10^6$ dmp)
2	24 May	0.13	0.13
3	25 May	2.20	2.23
4	26 May	7.48	9.81
5	27 May	2.30	12.11
7	29 May	10.84	22.95
9	31 May	3.34	26.29
12	3 June	1.31	27.60

phytoplankton. If only measured $^{14}CO_2$ data were used, the removal rate of crude oil from the water column might be underestimated. Similarly, decomposition in the sediment might also have occurred, and the DOC values were too low to be used for evaluation. Thus, the SPM ^{14}C data might provide a better source for estimating the removal rate. The integrated amount of SPM ^{14}C varied with time as two exponential functions separated on day 7 (Fig. 4). The whole transfer processes was divided into two steps to obtain the respective removal rates. In practice, the exponential function was expressed as

$$[2] \quad A_t = A_0 \exp(-kt)$$

where A_0 and A_t are the integrated SPM ^{14}C on day 0 and day t, respectively, and k is the removal rate. After logarithmic transformation, the following equation, is derived:

$$[3] \quad \ln(A_t) = \ln(A_0) - kt$$

in which the removal rate can be calculated from the slope of the line (Fig. 5). As a result, the removal rates determined for the two steps are:

$$k_1 = 0.37 \text{ d}^{-1} \ (r^2 = 0.99, n = 5)$$

and

$$k_2 = 0.17 \text{ d}^{-1} \ (r^2 = 0.99, n = 3)$$

Both estimates were within the 95% confidence intervals. The results indicated that there were significant differences in dynamics between the two steps. For oil discharge within a limit of 15 ppm, the removal rate was independent of the initial level of crude oil. The coefficients of correlation (r^2) of equation [3] were both 0.99, suggesting that the SPM ^{14}C was a good estimator of the rate.

The removal half-life, $T_{0.5}$, referred to the time required for one-half of the n-(1-^{14}C) hexadecane in SPM to sink to the bottom, escape to the atmosphere, or change to soluble forms. From equation [2], A_t can be set equal to one half of A_0 to obtain

$$[4] \quad T_{0.5} = \ln(2)/k$$

which gives $T_{0.5} = 2$ d and $T_{0.5} = 4$ d for the two steps respectively.

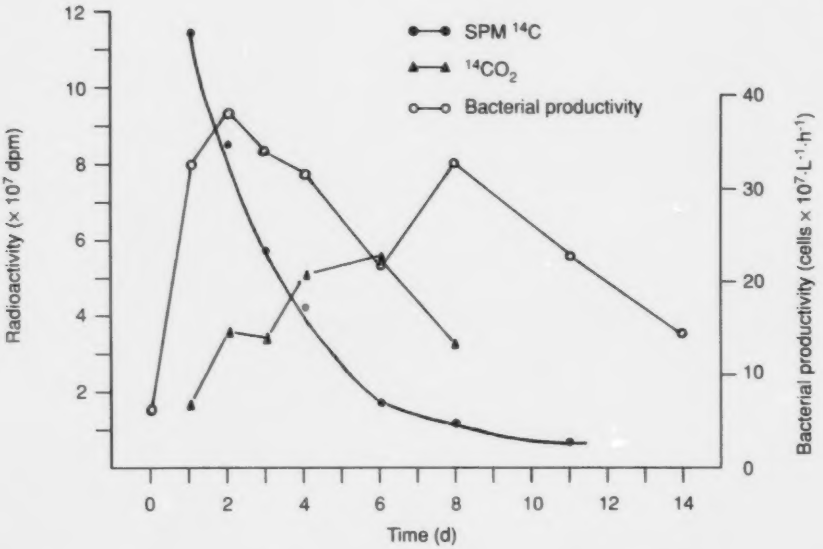

Fig. 4. Integrated amounts of suspended particulate matter (SPM) ^{14}C and $^{14}CO_2$ compared with bacterial productivity.

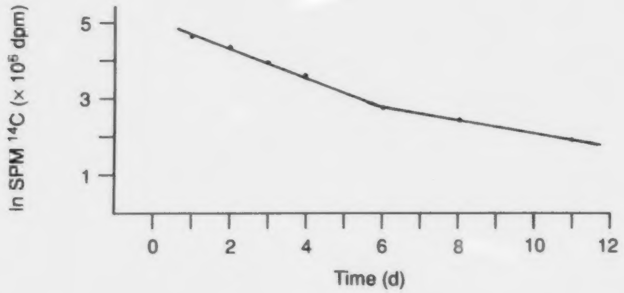

Fig. 5. Changes in log-transformed ^{14}C in suspended particulate matter (SPM).

Mechanism of oil-transfer process

As soon as crude oil was added to the enclosed ecosystem, the new carbon source would stimulate the activity of bacteria. Bacterial productivity increased rapidly from 64×10^6 cells·$L^{-1} \cdot h^{-1}$ on day 1 to 373×10^6 cells·$L^{-1} \cdot h^{-1}$ on day 3 (Table 2). At the same time, $^{14}CO_2$ production increased from 16.65×10^6 dpm to 35.11×10^6 dpm following the same trend. Although bacterial growth decreased from day 3 to day 7, the production of $^{14}CO_2$ did not slow down at all, reaching a peak of 54.53×10^6 dpm on day 7 (Fig. 4). While SPM ^{14}C decreased, DOC ^{14}C increased, reaching its maximum of 1.56×10^6 dpm on day 5, and sediment ^{14}C reached its maximum of 10.84×10^6 dpm on day 7.

Previous research on the fate of oil in the ocean has focused on the effects of bacterial attachment on organic particulates (Lee 1977). Results from the present study showed that at the 2.5-m layer on day 5, SPM ^{14}C retained on the 3-µm filter was about 70% of the total particulate observed (Table 5a). On day 7, the amount of SPM retained was 60% of the total (Table 5b). These observations indicated the formation of organic flocs. The first step was a rapid process. Biodegradation was found to be predominant. Peak $^{14}CO_2$ production appeared 4 d after the bacterial bloom, suggesting that it took only a few days for the bacteria to digest the oil droplets. Similar results were obtained in a study conducted in Canada.

The experimental ecosystem maintained a high level of dissolved silicate throughout the first removal step. This level of dissolved silicate decreased abruptly, however, at the beginning of the second step. There were considerable amounts of nitrate, nitrite, and phosphate during the first 3 d, thereafter, however, these amounts dropped to near background levels (Lin, Y., et al., this volume). Primary productivity decreased temporarily after the addition of crude oil, but increased on day 3, only to decrease later due to nutrient limitation (Chen et al., this volume).

The second removal step was a slow transfer process. On day 9, the size of SPM at the 2.5-m layer was mainly in the range of 0.45–3 µm, and only 27% of the material was >3 µm. The integrated amount of $^{14}CO_2$ production on day 9 was only 14.6% of the total tracer added. High concentrations of ammonium (3.9 $\mu g \cdot L^{-1}$) also appeared on day 9 (Lin, Y., et al., this volume) in contrast to other nutrients, suggesting a shift to a more reductive ecosystem. Although salinity remained relatively stable during the first step, it decreased significantly after day 11, perhaps due to changes in environmental conditions. Corresponding with the other peak of

Table 5a. Fractionation of suspended particulate matter ^{14}C ($dpm \cdot L^{-1}$) and dissolved organic ^{14}C ($dpm \cdot L^{-1}$) at a depth of 2.5 m.

		0.45 µm			3 µm			8 µm		
Day	Date	SPM	DOC	Total	SPM	DOC	Total	SPM	DOC	Total
2	24 May	8 939	16	8 955	—	—	—	—	—	—
3	25 May	7 082	31	7 113	—	—	—	—	—	—
4	26 May	4 700	31	4 731	—	—	—	—	—	—
5	27 May	2 775	359	3 134	1 840	811	2 651	435	358	793
7	29 May	1 359	4	1 363	772	542	1 314	107	176	283
9	31 May	1 135	0	1 135	305	122	427	187	161	348
12	3 June	582	—	—	154	145	299	159	179	338

Note: SPM, suspended particulate matter; DOC, dissolved organic carbon.

Table 5b. Normalization of the fraction of suspended particulate matter ^{14}C at a depth of 2.5 m.

Day	Date	0.45 µm	3 µm	8 µm
5	27 May	1	0.66	0.16
7	29 May	1	0.57	0.08
9	31 May	1	0.27	0.16
12	3 June	1	0.26	0.27

bacterial production on day 9, primary production also increased to a second maximum, but then decreased rapidly to lower levels. The process controlling the transfer of oil during the second step might be more complicated. The abnormally high $^{14}CO_2$ in the 2.5-m layer on day 12 (Table 2) probably resulted from decomposition of the oil found on the inner wall of the enclosure or contamination of the sampler.

Conclusions

It took about 2 weeks for the total transfer of crude oil dispersed with Corexit 9527. The whole process could be divided into two steps, each lasting about 1 week. The removal rate during the first step was 0.37 d^{-1}, with a removal half-life of 2 d. The first step was predominantly controlled by bacterial activity. The time required for the formation of organic flocs was estimated to be about 7 d, a delay of 4 d after the bacterial bloom. The removal rate for the second step was 0.17 d^{-1}, with a half-life of 4 d. The process for oil transfer during the second step was more complicated, probably because of changes in environmental conditions.

Residues of SPM ^{14}C, sediment ^{14}C, and DOC ^{14}C were 3.4, 12.4, and 1% of the total tracer added respectively. Production of $^{14}CO_2$ should be much higher than the measured 25% of the total tracer. The other "unrecovered" ^{14}C might be in crude oil that adhered to the inner wall of the enclosure, in some metabolic gases that escaped from the ecosystem, or in biodegradation products that are inextractable by CH_2Cl_2 such as carboxylic acids or polar organic matter.

Up to a limit of 15 ppm of oil addition and in the presence of dispersant, the marine enclosed ecosystem was able to transfer the oil totally by natural processes within 2 weeks.

Acknowledgments

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Cretney, W.J., MacDonald, R.W., Wong, C.S., Green, D.R., Whitehorse, B., Geesey, G.G. 1981. Biodegradation of a chemically dispersed crude oil. *In* Proceedings of the 1981 Oil Spill Conference, 2–5 March 1981, Atlanta, GA, USA. American Petroleum Industry, Washington, DC, USA. Publication 4334, 37–43.

Fuhrman, J.A., Azam, F., 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Marine Biology, 66, 109–120.

Gearing, J.N., Gearing, P.J., Wade, T., Quinn, J.G., McCarty, H.B., Farrington, J., Lee, R.F. 1979. The rates of transport and fates of petroleum hydrocarbons in a controlled marine ecosystem, and a note on analytical variability. *In* Proceedings of the 1979 Oil Spill Conference, 19–22 March 1979, Los Angeles, CA, USA. American Petroleum Institute, Washington, DC, USA. Publication 4308, 555–564.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parsons, T.R., Lalli, C.M., Wu, J. 1985. Microbial response to crude oil and Corexit 9527: SEAFLUXES enclosure study. Microbial Ecology, 11, 337–351.

Lee, R.F. 1977. Fate of petroleum components in estuarine waters of the southeastern United States. *In* Proceedings of the 1977 Oil Spill Conference. American Petroleum Institute, Washington, DC, USA. Publication 4284, 611–616.

______ 1980. Processes affecting the fate of oil in the sea. *In* Geyer, R.A., ed., Marine environmental pollution — 1: Hydrocarbons. Elsevier Scientific Publishing, Amsterdam, Netherlands, 337–351.

Parsons, T.R., Maita, Y., Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, UK.

Part IV

Other China–Canada Enclosure Experiments

Enclosure Study of Metal Release from Dredged Spoils and the Effect of Capping with Alluvial Materials

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Two types of enclosure experiments were conducted to investigate the fluxes of Cd and Pb from False Creek, BC, Canada, sediments into seawater. One experiment used a plastic bag enclosure 2.5 m in diameter × 15 m deep with a sediment pan carrying about 10 cm of sediment. The other experiment was performed using a special barge with five fibreglass tanks, each with a volume of 1 500 L, suspended in seawater to simulate various environmental conditions: (1) background control, no sediment added; (2) and (3) seawater in contact with False Creek sediments; (4) seawater in contact with alluvial "capping material"; and (5) seawater in contact with alluvial capping material (15 cm) over False Creek sediments. The Cd level in seawater in contact with False Creek sediment or alluvial capping material doubled in 5 weeks, and for capped sediments, increased by a factor of four, indicating the additive Cd fluxes from each type of sediment. In contrast, the alluvial capping material did not contribute Pb to seawater and, in fact, absorbed Pb. Seawater in contact with the capped False Creek sediment showed a 10-fold increase in Pb level compared with the control after 1 week, dropping to two-fold in 5 weeks. Seawater in contact with False Creek sediment alone showed a much higher increase of 20-fold after 1 week, dropping to five-fold in 5 weeks. This indicated that alluvial capping did not stop Pb flux effectively. Preliminary calculations showed that only 0.06% of the sediment-bound Cd and 0.001% of the sediment-bound Pb would be required to account for the seawater increases. The work emphasized the rudimentary nature of our present knowledge on sediment–seawater interaction for trace metals with significance for ocean dumping.

In preparation for dredging operations, a survey of benthic sediments (Brothers and Sullivan 1984) found that dredged spoils from False Creek, BC, Canada, were contaminated by high levels of Cd (1.5–40.0 $\mu g \cdot g^{-1}$). The levels of soluble Cd and soluble Pb in surface waters of False Creek were found to be 100 $ng \cdot kg^{-1}$ and 50–80 $ng \cdot kg^{-1}$ respectively (Stukas and Erickson 1983), only one fifth of the values indicated by laboratory release experiments using 200-L carboys of seawater with 1–100 pm of particulate matter in suspension for a closed system with constant stirring (Stukas 1983).

This paper describes enclosure experiments on fluxes of Cd and Pb using larger enclosures and the addition of an interface between the seawater and a benthic sediment layer to simulate sediment–seawater interaction in a more realistic way than can be achieved in a laboratory-scale study. Minicontrolled experimental ecosystem (CEE) experiments were conducted in August 1985 in Saanich Inlet, BC, in enclosures containing clean coastal waters and dredged spoils from False Creek and alluvial materials from the Fraser River estuary using 1500-L fibreglass cylinders lined with clean conventional polyethylene (CPE) plastic as described in Wong et al. (this volume). A mesoscale experiment was also conducted using two controlled ecosystem pollution experiment (CEPEX) plastic enclosures fitted with sediment pans (Wong et al., this volume) in which 70 000 L of seawater (2.5 m in diameter × 13–15 m deep) was in contact with 10–15 cm depths or 500–700 L of sediment. Time-series samples of seawater for soluble Cd, soluble Pb, and particulate organic carbon (POC) were obtained. Isotopic ratios of ^{206}Pb:^{207}Pb were analyzed using mass spectrometer and clean-room techniques (Wong et al. 1983) to identify the sources of Pb in the enclosure i.e., gasoline derived or sediment derived.

The objectives of the study were to quantify the release rates of metals from the organic-rich False Creek sediment and to investigate the efficiency of capping the contaminated sediment with clean alluvial materials.

Methods

The portable marine enclosures system (PMES), a catamaran barge supporting up to six fibreglass cylinders (1 m in diameter × 2 m deep), was launched in Patricia Bay, Saanich Inlet, BC. Each of the cylinders was filled with 1500 L of seawater pumped from a depth of 10 m in Saanich Inlet, taking special precautions to minimize exposing the collected water to atmospheric Pb contamination (Stukas and Wong 1981; Wong et al. 1983). Peristaltic pumps were employed, using a short length of acid-rinsed Tygon or silicone tubing within the pump connected to 20-mm internal diameter, acid-cleaned CPE tubing, positioned with a 10-kg stainless steel weight located 1.5 m above the intake port, at a combined pumping rate of about 50 $L \cdot min^{-1}$. The marine sediment pan enclosure system (MSPES), a CEPEX-type woven polyethylene bag (2.5 m × 16 m) fitted with a steel pan to hold sediment, was filled with seawater by scuba divers from a depth of 15 m (Menzel and Case 1977). Seventy percent of the bag was filled in this manner, the balance being filled by peristaltic pumping.

Seawater samples were collected for chemical analysis using a modified vacuum intercept pumping system (VIPS), which is capable of preserving the integrity of ultralow levels of Pb in seawater. The sampled seawater was aspirated directly into an evacuated Plexiglas chamber housed in a portable Class-100 clean-air hood. Acid-cleaned CPE bottles were used to store the samples. Three to five volumes of water were flushed through the tubing (7 mm in diameter) before sampling.

Between sampling in the cylinders of the PMES, the sampling tube and its Teflon weight were flushed and stored in 0.1% HNO_3 (Seastar ultrapure double quartz-distilled) in Milli-Q water. When not in use, the sampler was stored in 0.1% HNO_3. Seawater samples were triple bagged in CPE under Class-100 clean-air conditions and stored in a cooler at 4°C before extraction and analysis.

Sediment for the experiment was collected from dredges of 3 m^3 in False Creek in Richmond Marina. Surface sediment at the head of False Creek was collected in ten 200-L drums lined with CPE bags. To preserve the anoxic state, air was excluded from the bagged sediments. The clean alluvial materials were collected from a 3-m deep hole dug below datum by a 3-m^3 dredge in Richmond Marina and stored in two 200-L CPE-lined drums. Sediments were shoveled into the fibreglass cylinders and into the sediment pan, and were then subsampled for chemical analysis. Five 10-g and five 100-g samples were collected from each of the fibreglass cylinders. From the 2.5-m diameter sediment pan, 16 samples were obtained from the centre of each square in a 4 × 4 grid laid out on the sediment surface. CPE-bagged samples were stored in a frozen state until they were analyzed.

Chemical analyses

Lead and cadmium in the seawater samples were extracted using 0.001% dithizone in chloroform and were analyzed using the "double spike" version of the isotope dilution mass spectrometry (IDMS) technique (Stukas and Wong 1983). Using two isotopic spikes provides simultaneous yield and concentration, thereby compensation for yield problems. Sampling duplicates and analytical duplicates used in this study were generally within an analytical precision of about ± 1 $ng \cdot kg^{-1}$ for Cd and about ± 0.5 $ng \cdot kg^{-1}$ for Pb for seawater containing Cd at about 65 $ng \cdot kg^{-1}$ and Pb at 15 $ng \cdot kg^{-1}$. Dithizone blanks of 0.005 ± 0.003 ng Cd and 0.23 ± 0.04 ng Pb were obtained. Detection limits using this method were about 0.02 $ng \cdot kg^{-1}$ for Cd and 0.2 $ng \cdot kg^{-1}$ for Pb for seawater samples of 200–500 g. The isotopic composition of Pb in seawater was determined for unspiked subsamples of 200–500 g of seawater extracted using the above method. Lead ratios were normalized to the common Pb isotopic standard NBS 981.

Particulate organic carbon (POC) was measured in aliquots filtered onto precombusted (450°C, 2 h), 47-mm diameter Whatman GF/C filters. The filters were placed in precombusted glass petri dishes, dried in an oven at 60°C for 24 h, and stored in a frozen state. Carbonate was removed by exposing the filtered samples, just before analysis, to hydrochloric acid vapour for 8 h in a closed container. The filters were then dried in an oven at 50°C for 24 h. The POC content was analyzed using a Perkin-Elmer 240 elemental analyzer at a combustion temperature of 750°C.

Observations and discussion

For the metal-release experiment, five fibreglass tanks were used: control, seawater only; two tanks of seawater plus False Creek sediment; seawater plus False Creek sediment capped with alluvial materials; and seawater plus alluvial materials. There was little concentration gradient with depth for four sampling depths within the 2-m cylinder, indicating homogeneity due to mixing as a result of daily heating and cooling. Thus, it was sufficient to use only the middepth concentrations of Cd and Pb to plot their release into seawater with time (Figs 1 and 2).

In the case of Cd, seawater in contact with sediments or alluvial materials showed an initial drop of 20–70% of the value in the control for the first sampling after 3 d. Then, concentrations of soluble Cd showed monotonically increasing levels with time until the end of the experiment after 5 weeks. The initial drop in Cd was due to absorption onto suspended particles, consistent with observations by

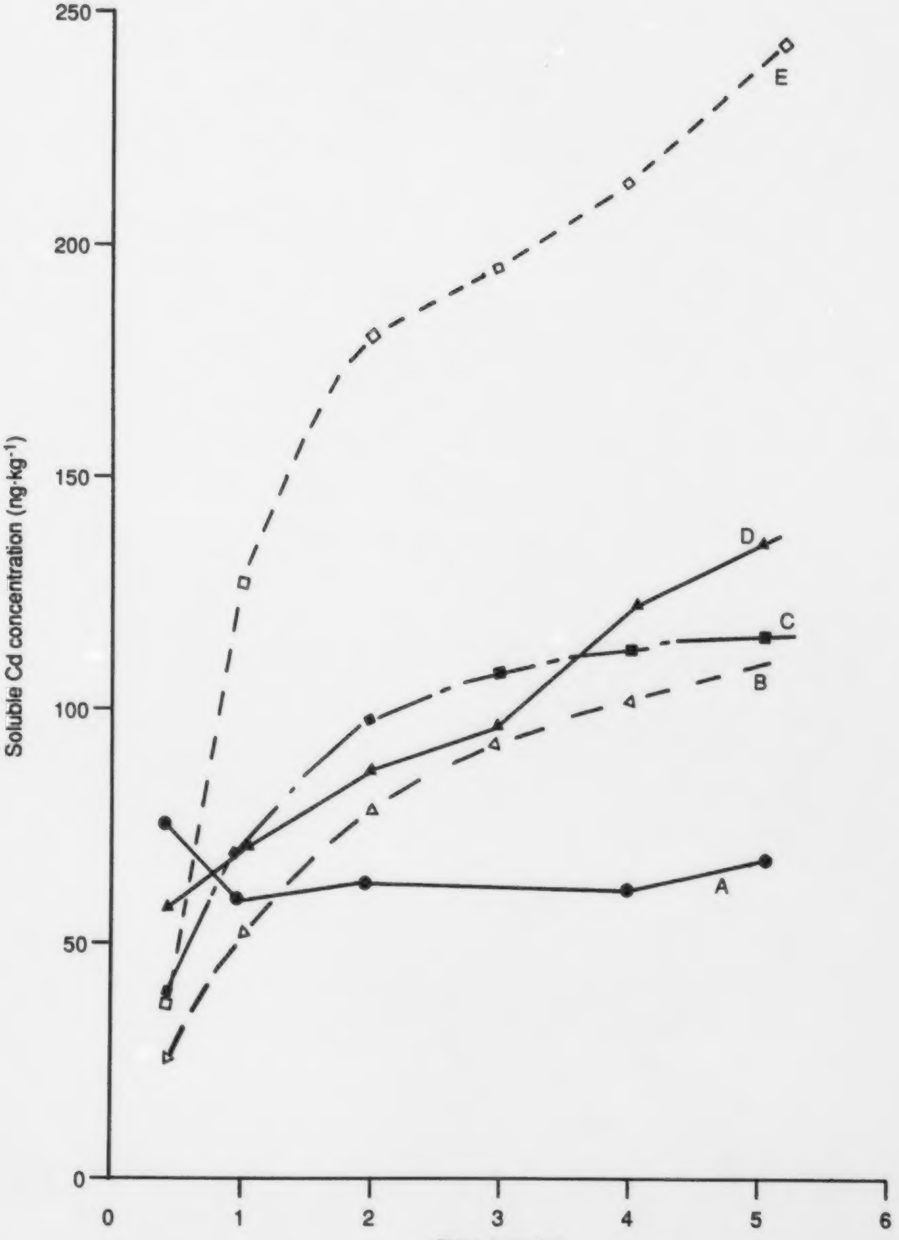

Fig. 1. Release of soluble Cd from sediment in contact with seawater in fibreglass cylinders: (A) control, without sediment; (B and C) False Creek sediment; (D) alluvial capping material; and (E) False Creek sediment capped by 10–15 cm of alluvial material.

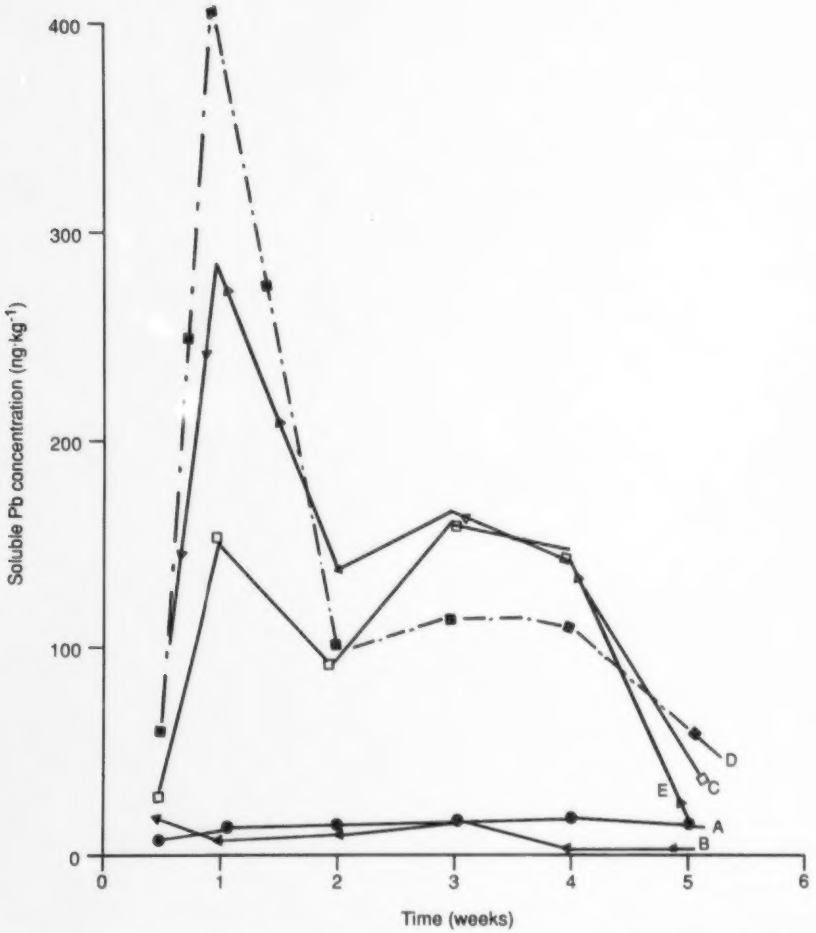

Fig. 2. Release of soluble Pb from sediment in contact with seawater in fibreglass cylinders: (A) control, without sediment; (B and C) False Creek sediment; (D) alluvial capping material; and (E) False Creek sediment capped by 10–15 cm of alluvial material.

Stukas (1983) for laboratory-scale studies. The alluvial capping materials showed the same magnitude of release as the organic-rich False Creek sediment. These alluvial materials were considered to be clean, with less than 0.3 $\mu g \cdot g^{-1}$ for Cd. The largest increase occurred in the tank containing both False Creek sediment and alluvial capping materials, with 240 $ng \cdot kg^{-1}$ for Cd in seawater after 5 weeks, equal to the combined final values of 110 $ng \cdot kg^{-1}$ for Cd for the False Creek sediment tank and of about 135 $ng \cdot kg^{-1}$ for Cd for the alluvial material tank. Thus, capping with 10–15 cm of clean alluvial material did not prevent the flux of Cd from the contaminated sediment.

The behaviour of Pb release from the sediments was different. In contrast with an initial drop for Cd, a sharp rise in Pb levels above background values occurred in the tanks with False Creek sediments during the period from day 3 to 1 week. However, a sharp decrease in dissolved Pb occurred in these tanks during the 2nd week, followed by a secondary release until 3–4 weeks, then another decrease, so that, after 5 weeks, Pb concentrations in the seawater approached background levels of about 15 $ng \cdot kg^{-1}$. Lead levels in the tank containing False Creek sediment capped by alluvial sediment showed the closest approach to background levels. The release pattern for the contaminated sediment was similar to that for mine tailings in seawater (Hoff et al. 1982). The alluvial materials did not perturb Pb levels in the water column because the tanks with seawater and with seawater and alluvial materials remained at low Pb levels throughout the experiment. Capping with alluvial materials did not prevent Pb release from contaminated sediment into seawater except during the 1st week, when a significant increase in Pb for False Creek sediment occurred. For the control seawater, Pb concentrations of 11–18 $ng \cdot kg^{-1}$ were typical for clean seawater in Saanich Inlet (Stukas and Wong 1981), implying little or no contamination during filling and sampling processes for the experiment.

The release of a very small fraction of the metals bound in the sediment or alluvial materials could account for the levels observed in these release experiments. Only 0.8% of the total alluvial-bound Cd or 0.06% of the total Cd bound in the False Creek sediment would be required to yield levels of 120 $ng \cdot kg^{-1}$ for Cd in seawater. Leaching from a surface layer of sediment of only 0.06 mm would be sufficient. Lead in the alluvial material was only 1–15 $\mu g \cdot g^{-1}$ and 200–800 $\mu g \cdot g^{-1}$ in the False Creek sediments, which can potentially yield 1 000–1 500 $\mu g \cdot kg^{-1}$ and 20 000–80 000 $\mu g \cdot kg^{-1}$ of Pb respectively. About 0.005–0.02% of the contaminated sediment would produce the maximum Pb levels observed in the experiment. As little as 0.001% of the sediment-bound Pb would account for the average levels in the enclosures.

Soluble Cd profiles in seawater showed different variations with time for the upper 5 m and lower 10 m (Fig. 3). In the first 2 weeks, Cd levels were similar to the levels of 60–65 $ng \cdot kg^{-1}$ observed in control sea water. After 4 weeks, the Cd levels decreased in the surface layer to about 20 $ng \cdot kg^{-1}$, possibly due to settling of suspended matter and phytoplankton. In the bottom of the water column, Cd levels increased with depth and with time between days 4 and 22. An anomalously low Cd level on day 16 occurred after a period of calm between days 11 and 16, without the usual stirring by moderate winds and wave heights of 10–20 cm that persisted during other periods of the experiment. On day 29, the collapse of the bag below 8 m halted sampling in the lower water column.

Soluble Pb profiles followed the same changes as for Cd (Fig. 4). At the beginning of the experiment, Pb levels were similar to background levels observed in the control at 6-13 $ng \cdot kg^{-1}$ for surface waters. Lead levels were constant at 20–30 $ng \cdot kg^{-1}$ in the upper 10 m and rose quickly to 75 $ng \cdot kg^{-1}$ at 13 m, toward the seawater–sediment interface. By day 11, Pb levels changed to 250 $ng \cdot kg^{-1}$ at 10 m and about 600 $ng \cdot kg^{-1}$ at 14 m. During the calm period, the Pb level dropped as for Cd and POC, which decreased from a high of 7 000 $\mu g \cdot L^{-1}$ to about 1 000 $\mu g \cdot L^{-1}$ for days 11 and 16 respectively. On day 22, levels of Pb were high throughout the bag, with the highest value in the high POC turbid layer near the bottom of the sediment pan. The isotopic ratio of ^{206}Pb:^{207}Pb in the control seawater at the start of the experiment was 1.148:1, a value identical to that for Pb in gasoline (Stukas and Wong 1981), reinforcing the notion that most coastal waters contain Pb of predominantly gasoline origin. Isotopic ratios for Pb and in the bag with False Creek sedi-

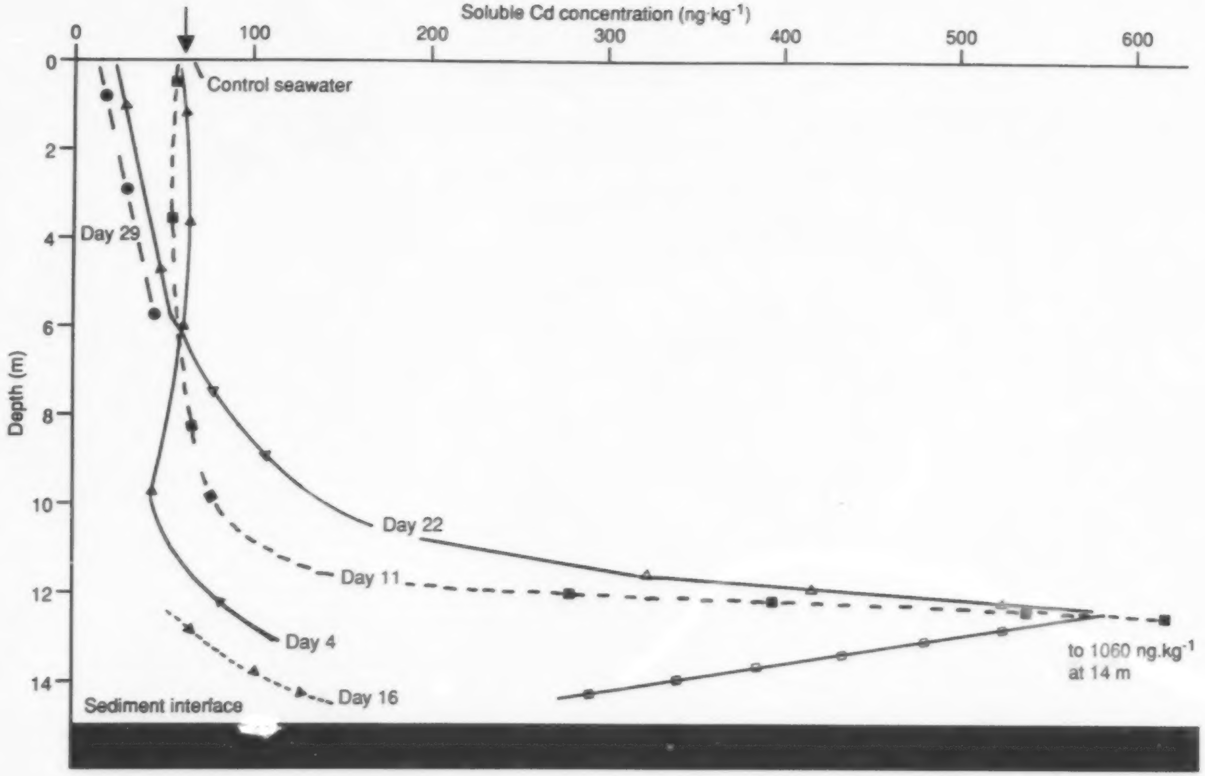

Fig. 3. Release of soluble Cd from False Creek sediment in contact with seawater in a CEPEX bag fitted with a sediment pan.

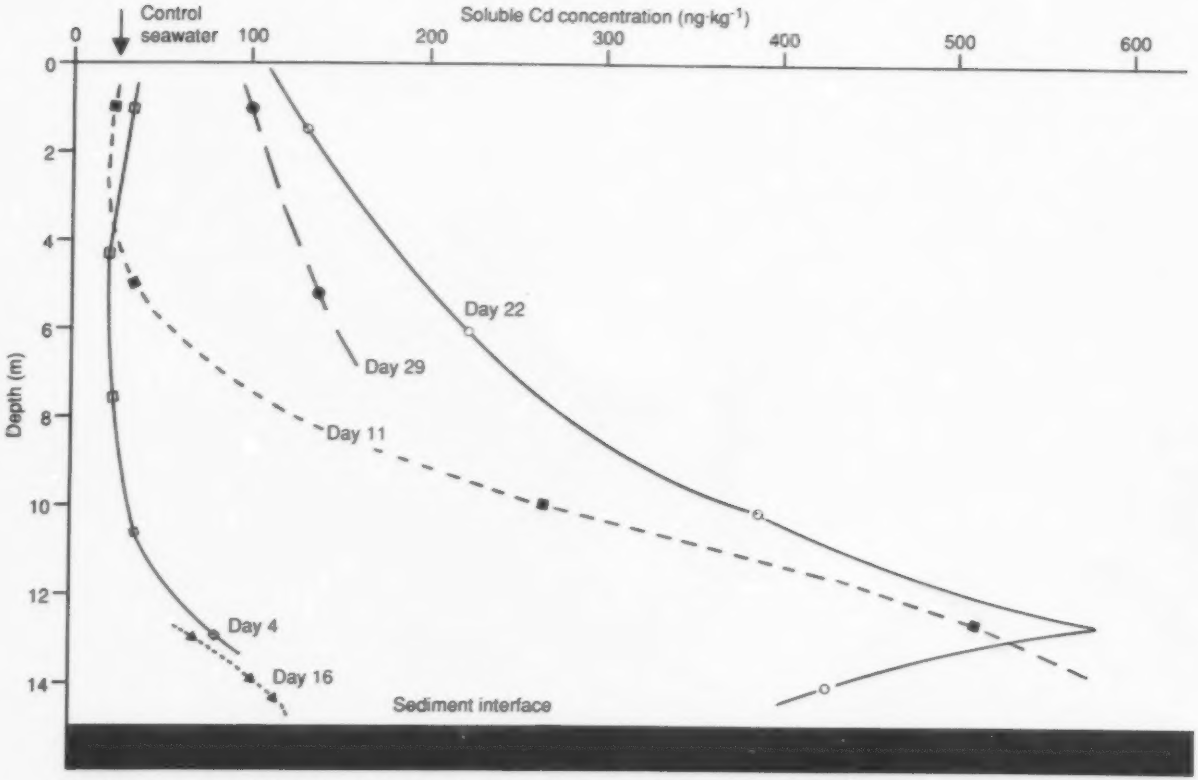

Fig. 4. Release of soluble Pb from False Creek sediment in contact with seawater in a CEPEX bag fitted with a sediment pan.

ment converged with time and with depth to a final value of 1.133 for days 22 and 29. Lead leached out of the False Creek sediment overwhelmed the gasoline Pb signature during the period.

Conclusions

Two types of enclosures were shown to be effective in the investigation of seawater–sediment interactions. They are the portable marine enclosures system (PMES), a catamaran barge supporting up to six fibreglass cylinders (1 m in diameter × 2 m deep), and the marine sediment pan enclosure system (MSPES), a CEPEX-type woven polyethylene bag (2.5 m in diameter × 16 m deep) connected to a steel pan to hold sediment.

Cadmium levels in seawater in contact with sediment showed an initial drop of 20–70% after 3 d, then a monotonic increase with time during the 5-week study using the PMES.

Lead levels in seawater in contact with sediment, in contrast with Cd, showed a sharp rise to a maximum in 10 d, decreasing to a plateau over the 2–4 week period, and finally dropping to values approaching background levels observed in the seawater control, using the PMES.

Clean alluvial materials were ineffective in preventing metal fluxes of Cd and Pb from escaping from contaminated sediment into overlying waters, at least for an alluvial material thickness of 10–15 cm.

Very small quantities of metals released from the sediment, in the range of 0.001–0.06% of the amounts bound by contaminated sediment, could account for the increase in metal levels in seawater.

The mesoscale experiment using the MSPES showed that Pb and Cd were released in a layer of turbid bottom water with high POC, with suspended matter being kept in the water above the sediment–seawater interface by wave and wind action. Metal levels dropped drastically during a period of calm at the experimental site in Saanich Inlet.

Brothers, D.E., Sullivan, D.L. 1984. False Creek benthic sediment survey, 1982/83. Environmental Protection Services (Pacific Region), Vancouver, BC, Canada. Regional Program Report 84-08, 48 pp.

Hoff, J.T., Thompson, J.A.J., Wong, C.S. 1982. Heavy metal release from mine tailings into sea water — a laboratory study. Marine Pollution Bulletin, 13, 283–286.

Menzel, D.M., Case, J. 1977. Concept and design: controlled ecosystem pollution experiment. Bulletin Marine Science, 27, 1–7.

Stukas, V. 1983. Release of cadmium and lead from False Creek sediments: a laboratory study. Institute of Ocean Sciences, Sidney, BC, Canada. Technical Report, DSS File 06SB.FP941-2-0965, 21 pp.

Stukas, V., Erickson, P. 1983. Total and dissolved Cu, Cd, Pb, Zn and Hg concentrations in surface sea water of False Creek, BC. BC Place Ltd and Environmental Protection Services, Vancouver, BC, Canada. Unpublished report.

Stukas, V., Wong, C.S. 1981. Stable lead isotopes as a tracer in coastal waters. Science, 21, 1424–1427.

______ 1983. Accurate and precise analysis of trace levels of Cu, Cd, Pb, Zn, Fe and Ni in sea water by isotope dilution mass spectrometry. *In* Wong, C.S., ed., Proceedings of a NATO Advanced Research Institute Conference on Trace Metals in Sea Water, held 30 March–3 April 1981 in Erice, Sicily, Italy. Plenum Press, New York, NY, USA. 513–536.

Wong, C.S., Kremling, K., Riley, J.P., Johnson, W.K., Stukas, V., Berrang, P.J., Erickson, P., Thomas, D., Petersen, H., Imber, B. 1983. An intercomparison of sampling devices and analytical techniques using sea water from a CEPEX enclosure. *In* Wong, C.S., ed., Proceedings of a NATO Advanced Research Institute Conference on Trace Metals in Sea Water, held 30 March–3 April 1981 in Erice, Sicily, Italy. Plenum Press, New York, NY, USA. 175–193.

Releasing Experiment of Mine Tailings from Alice Arm, BC, Canada

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This 1984 study was conducted in Alice Arm, BC, Canada, using a portable marine enclosure platform (PMEP). After the addition of mine tailings to the enclosed water column, changes in the concentrations of dissolved and particulate Cu, Pb, Zn, Cd, Fe, and Ni were determined. The effects of mine tailings on primary productivity, chlorophyll a, particulate organic carbon, and the biological removal of trace metals in the water column are discussed. Primary productivity and chlorophyll concentrations were not affected by the addition of mine tailings in low concentrations (tank PCEE-10), but were inhibited by the addition of mine tailings in high concentrations (tank PCEE-100). The phytoplankton bloom was also delayed in PCEE-100. About 96 h after the addition of mine tailings, primary productivity and chlorophyll concentrations in PCEE-100 were only 14 and 15% of the respective peak values observed during a phytoplankton bloom in PCEE-10. Concentrations of dissolved Fe and Zn declined significantly from initial values before the addition of mine tailings. A small increase in the concentration of dissolved Cu was observed in both tanks. Concentration of dissolved Cd and Pb also increased with time as Cd and Pb were released from the mine tailings. In PCEE-10, dissolved Ni stayed at the same level before and after the experiment, whereas in PCEE-100, it showed a significant increase. The different behaviours of trace metals were explained in terms of flocculation and sedimentation of Fe colloids, organic complexing, scavenging and settling of biogenic detritus, and adsorption of trace metals on hydrous oxides.

The behaviour of mine tailings disposed of in the ocean is gradually being understood. In seawater, the chemical properties of mine tailings are rather unstable. Observations in the laboratory reveal that the addition of mine tailings to seawater alters the concentrations of trace metals due to interaction of the mine tailings and the seawater (Hoff et al. 1982). Many scientists also recognize the environmental impacts associated with disposing of mine tailings in the ocean (Waldichuk 1978; Waldichuk and Buchanan 1980). Marine enclosed ecosystems have been used to study the effect of mine tailings on concentrations of trace metals in seawater (Wong et al. 1983). The controlled enclosures facilitate studying interactions among disposed mine tailings, seawater, and the marine environment.

Mine tailings are complex materials and, as they come into contact with seawater, many chemical reactions — such as ion exchange, dissolution, sorption, desorption, hydrolysis, and sedimentation — occur. This report focuses on three topics: first, variations in concentrations of Cu, Pb, Zn, Cd, Fe, and Ni after the addition of mine tailings; second, effects of mine tailings on primary productivity, and concentrations of chlorophyll and particulate organic carbon (POC); and third, the role of organisms on the removal of trace metals in the water column.

Methods

The experiment was conducted between 28 June and 5 July 1984, in Alice Arm, BC, Canada. A portable marine enclosure platform (PMEP) (Wong et al., this volume) was designed for easy transportation to and assembly at the experimental site. The platform consisted of two cylindrical fibreglass tanks (0.9 m in diameter × 1.8 m deep), an aluminum frame, and two fibreglass floats. A peristaltic pump was used to fill the tanks with 1 100 L of seawater drawn from the 50-m layer of station AA5. The tanks were designated as PCEE-10 and PCEE-100, which were treated with 10 and 100 ppm of the mine tailings respectively. A cubical polyethylene tank with a volume of 26 L was used as the control (PCEE-C).

Time-sequence samples were taken at 0, 1.5, 4, 10.5, 25, 45, 70.5, and 93 h. A polyethylene tube pretreated with acid was used to obtain water samples integrally from the whole water column. Filtration of the water samples was carried out in the ventilation cabinet on board the ship. The samples were filtered through an acid-treated membrane filter (0.45-µm Nuclepore). Both the filtrates and the filters were stored at 4°C for chemical analyses later in the ultraclean laboratory at the Institute of Ocean Sciences (Wong et al. 1983).

Basically, the method used to determine dissolved trace metals in the seawater followed that of Danielsson et al. (1978). In brief, trace metals in the seawater were extracted with a mixture of 1% APDC/DDDC and Freon, then back-extracted in a 5% solution of Milli-Q distilled water and HNO_3. Each filter and its retained particles were dried at 60°C for 24 h. The filter and associated particles were placed in a Teflon bomb using the method of Eggiman and Betzer (1976) together with 0.75 mL of redistilled HCl and left for 30 min at room temperature. Another 0.25 mL of redistilled HNO_3 and 0.05 mL of HF were added in the digestion bomb, which was then closed and placed in a water bath at 90–100°C for 3 h. The digested solution was diluted with 25–30 mL of metal-free water for subsequent analyses. An atomic absorption spectrophotometer (Perkin-Elmer 503 model equipped with an HGA-400 graphite furnace) was used to determine the concentrations of several trace metals in the extracts and the digested solutions. Table 1 gives the blank determinations and repeatability of the analyses.

A modified method of Strickland and Parsons (1972) was used to analyze the POC and particulate organic nitrogen (PON) (Wong et al. 1983). The seawater

Table 1. Blank determinations and deviation of analyses of dissolved and particulate trace metals.

	Cu	Pb	Zn	Cd	Fe	Ni
Dissolved metals blanks	3.5	0.9	33	0.4	35	27
Percentage deviation	6	13	10	10	20	7
Particulate metals blanks	0.6	0.2	5.4	0.1	43	3.7

sample was filtered through a precombusted (500°C for 4 h) fibreglass filter (Whatman GF/C). The materials retained on the filter were washed with 3% NaCl, dried at 60°C, and analyzed with a CHN elemental analyzer (Perkin-Elmer 240) at 750°C.

Results

Table 2 lists the concentrations of nutrients (nitrate, phosphate, and silicate), chlorophyll, POC and PON, and the primary productivity. Figures 1-3 illustrate the variations of these parameters with time.

Table 2. Variations of nutrients (nitrate, phosphate, and silicate), particulate organic carbon (POC), and particulate organic nitrogen (PON), chlorophyll (CHL), and primary productivity (PP) in seawater.

Time (h)	NO_3 (µ*M*)	PO_4 (µ*M*)	SiO_3 (µ*M*)	POC ($\mu g \cdot L^{-1}$)	PON ($\mu g \cdot L^{-1}$)	CHL ($mg \cdot m^{-3}$)	PP (mg C·$m^{-3} \cdot h^{-1}$)
				PCEE-10			
0	13.8	1.56	31.3	81.5	13.6	0.21	0.90
2	13.8	2.66	30.3	—	—	—	0.84
6.5	—	—	—	—	—	0.25	0.83
23.5	13.8	1.64	29.8	149	19.8	0.41	2.53
47.5	13.2	1.46	26.5	218	44.5	3.75	22.7
73	4.2	0.86	18.8	725	142	18.9	80.2
95	2.4	0.61	15.5	693	126	16.7	48.4
99	—	—	—	—	—	14.4	—
				PCEE-100			
0	13.8	1.55	22.1	89.8	10.4	0.18	1.18
2	13.8	2.66	30.3	—	—	—	0.84
6.5	—	—	—	—	—	0.08	0.36
23.5	13.8	1.86	35.0	175	17.3	0.11	0.32
47.5	14.1	1.65	28.6	94.5	16.3	0.25	0.76
73	13.8	1.50	29.0	171	19.3	0.96	3.64
95.5	12.8	1.28	27.9	153	33.5	2.87	11.3
99	—	—	—	—	—	3.68	--
				PCEE-C			
0	14.1	1.58	30.3	60.9	11.6	0.20	0.24
6.5	—	—	—	—	—	0.14	0.37
10.5	—	—	—	—	—	—	0.48
23.5	14.2	1.63	30.1	93.5	16.5	0.45	0.25
27.5	—	—	—	—	—	—	2.95
47.5	14.0	1.51	17.8	133	26.7	1.38	2.28
73	9.5	1.07	28.1	358	81.5	5.54	12.8
77	—	—	—	—	—	7.14	33.0
83	—	—	—	—	—	13.6	11.3
95.5	0.1	0.47	12.9	845	142	21.7	17.8
99	—	—	—	—	—	12.5	20.0

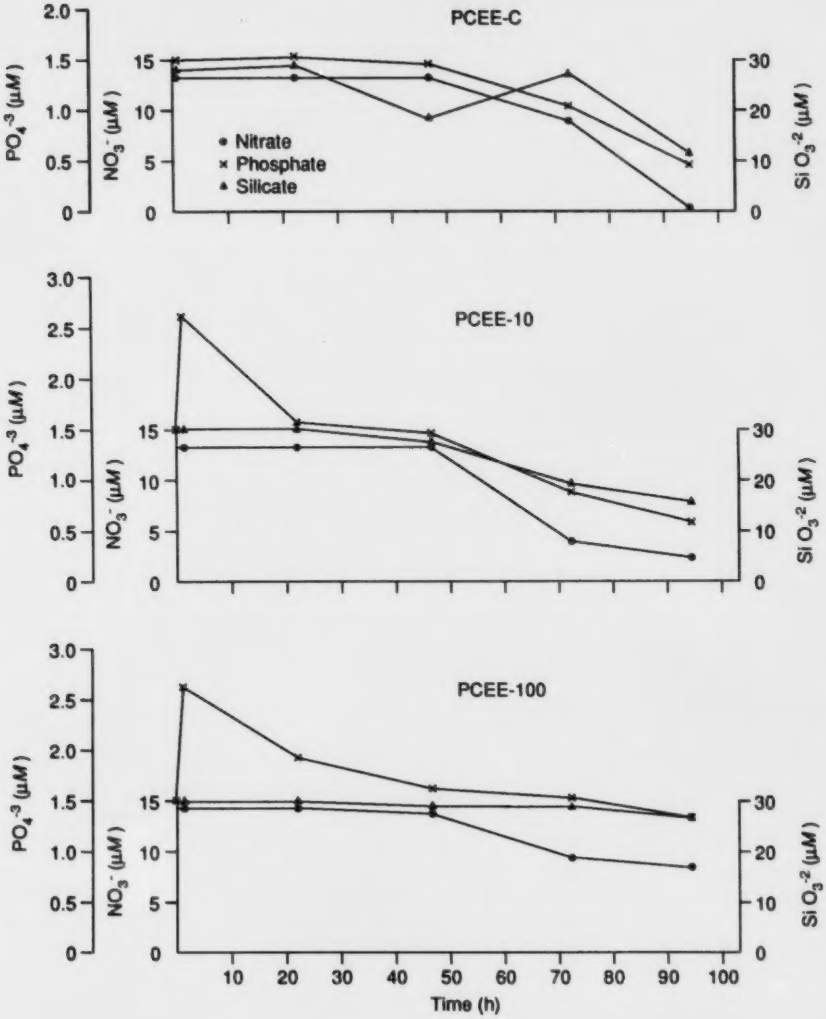

Fig. 1. Variations of nitrate, phosphate, and silicate in PCEE-C, PCEE-10, and PCEE-100.

Before the addition of mine tailings, primary productivities in all of the tanks were fairly low, about 2.6 mg $C{\cdot}m^{-3}{\cdot}h^{-1}$, and chlorophyll concentrations were less than 0.45 $mg{\cdot}m^{-3}$. Concentrations of nitrate, phosphate, and silicate remained relatively stable during the first 23 h of the experiment. Thereafter, primary productivities and chlorophyll concentrations in PCEE-C and PCEE-10 increased significantly, whereas those in PCEE-100 showed only a small change. At the same time, nitrate concentrations in PCEE-C and PCEE-10 began to decrease, but the nitrate concentration in PCEE-100 remained about the same.

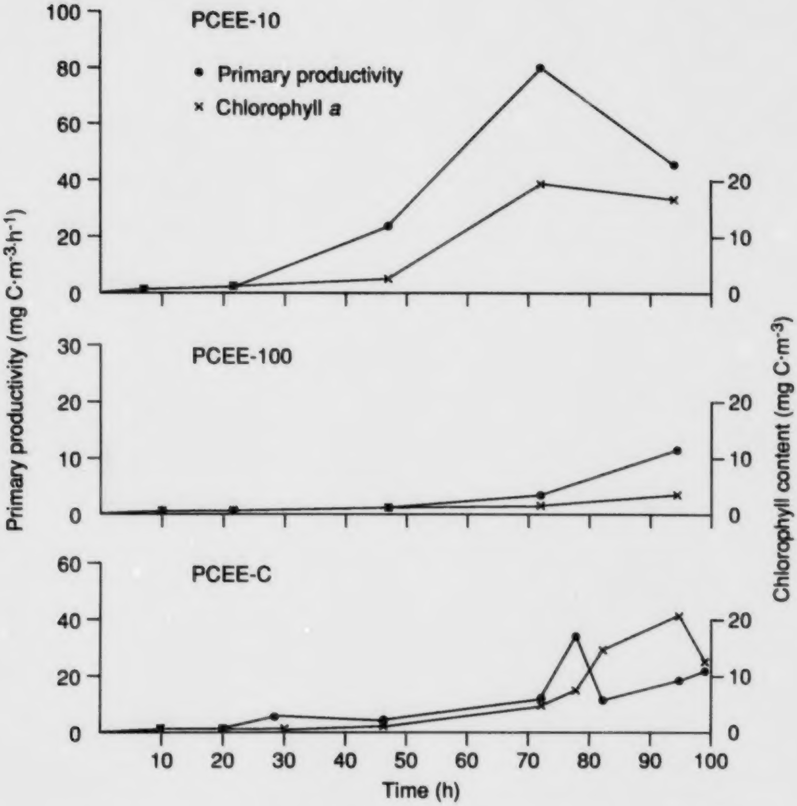

Fig. 2. Variations of primary productivity and chlorophyll content in PCEE-C, PCEE-10, and PCEE-100.

By the end of the experiment, nitrate concentrations in PCEE-C and PCEE-10 had decreased to 0.1 and 2.4 μ*M*, respectively, whereas that in PCEE-100 still remained at a relatively high level of 12.8 μ*M*. About 73 h after treatment, primary productivity and chlorophyll concentrations in PCEE-10 reached their peak values of 80.2 mg $C{\cdot}m^{-3}{\cdot}h^{-1}$ and 18.9 $mg{\cdot}m^{-3}$ respectively. In PCEE-C, primary productivity increased to a maximum of 33 mg $C{\cdot}m^{-3}{\cdot}h^{-1}$ 77 h after treatment, whereas the maximum chlorophyll concentration of 21.7 $mg{\cdot}m^{-3}$ was obtained 95.5 h after the addition of mine tailings. After the planktonic blooms, both primary productivities and chlorophyll concentrations decreased from their peak values. In PCEE-100, the primary productivity and chlorophyll concentration increased gradually at very low levels until 47 h, after which time the rates of increase became much higher. At the end of the experiment (95.5 h), the primary productivity was 11.3 mg $C{\cdot}m^{-3}{\cdot}h^{-1}$ and the chlorophyll concentration was 2.87 $mg{\cdot}m^{-}3$.

Concentrations of dissolved and particulate trace metals (Cd, Cu, Fe, Ni, Pb, and Zn) and suspended matter are given in Table 3. Figures 4–6 depict the variations of these concentrations with time. In PCEE-10, 69% of the suspended matter was lost

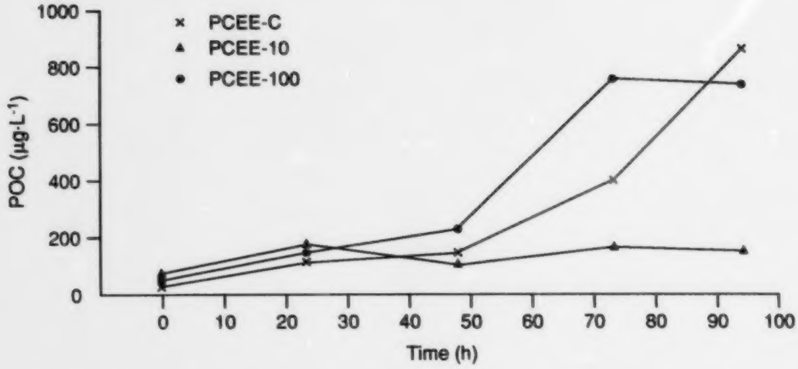

Fig. 3. Variations in the concentration of particulate organic carbon (POC) in PCEE-C, PCEE-10, and PCEE-100.

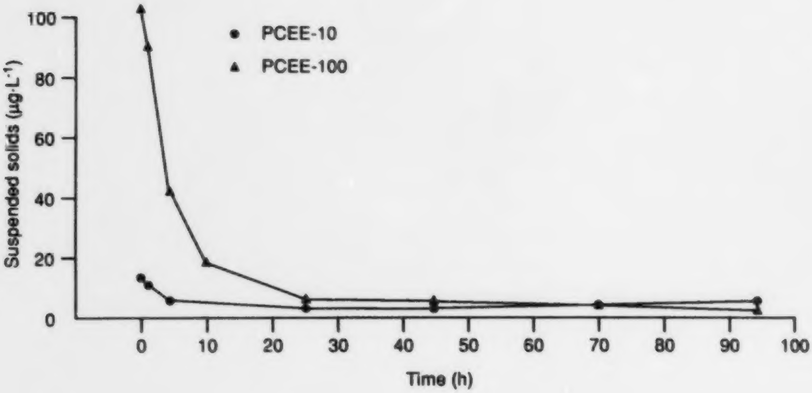

Fig. 4. Variations in the concentration of suspended particles in PCEE-10 and PCEE-100.

during the first 25 h, and 27.8% was lost between 25 h and 93 h; whereas in PCEE-100, 93% was lost during the first 25 h, but only 4% during the next 70 h.

In PCEE-10, the concentration of dissolved Cu increased from the background level of 187 ng·kg^{-1} to 210 ng·kg^{-1} (an increase of 15%) and then stayed at a steady level, averaging 237 ng·kg^{-1} (sd = 9%; $n = 7$). After the addition of mine tailings, concentrations of particulate Cu increased during the first 10.5 h; double in PCEE-10 and 1.5 times in PCEE-100. Thereafter, concentrations of particulate Cu in these two tanks decreased gradually.

The level of dissolved Ni in PCEE-10 did not change substantially after treatment. The average concentration was 799 ng·kg^{-1} (sd = 5%; $n = 8$). In PCEE-100, the concentration of dissolved Ni was nearly twice the background level of 717 ng·kg^{-1}, with a final value of 1 369 ng·kg^{-1}. Concentrations of particulate Ni in the two tanks increased initially and then decreased with time.

Table 3. Variations in the concentrations of dissolved (DTM, $ng \cdot kg^{-1}$) and particulate (PTM, $\mu g \cdot g^{-1}$) trace metals in seawater.

Time (h)	Cu		Pb		Zn		Cd		Fe		Ni		Suspended particles ($mg \cdot kg^{-1}$)
	DTM	PTM	DTM	PTM	DTM	PTM	DTM	PTM	DTM	PTM	DTM	PTM	
							PCEE-10						
—[a]	187	53	24.6	293	1.26	—	46.7	0	201	47	—	812	0.72
0	210	66	57.4	238	0.93	476	66.1	8.2	156	56	778	—	13.0
1.5	245	84	74.4	308	1.03	530	51.6	10.5	243	29	823	73	11.5
4	222	91	60.2	296	1.12	497	62.4	8.5	226	36	828	86	6.7
10.5	222	101	28.3	266	1.10	360	76.5	9.0	256	35	853	51	5.2
25	241	91	46.4	210	1.13	286	79.1	6.5	110	36	750	42	4.0
45	270	63	59.6	212	1.45	81.7	93.1	4.5	60	26	739	27	4.5
71	—	56	—	169	—	144	—	3	—	23	—	32	3.9
93	247	38	41.9	149	0.49	133	97.4	1.4	54	9.7	810	18	5.4
							PCEE-100						
—[a]	333	26	182	364	1.63	621	56.3	0	176	27	717	49	1.26
0	321	68	451	200	2.22	272	77.3	9.9	295	8.7	1 106	47	103
1.5	297	90	447	226	1.75	297	65.0	11.9	216	9.4	1 051	44	89.9
4	395	101	184	158	1.37	306	62.5	13.1	193	18	1 027	48	41.3
10.5	369	173	437	312	1.53	258	75.0	10.6	111	23	1 174	55	17.1
25	363	104	441	440	1.39	299	70.5	10.0	79	32	1 483	95	7.1
45	375	68	152	342	1.15	273	78.5	10.2	53	28	1 332	46	6.5
71	—	63	—	284	—	234	—	4.5	—	30	—	50	3.3
93	377	105	180	340	1.20	250	81.6	4.3	61	21	1 369	38	2.6

[a] Before additions.

Initially, the concentration of dissolved Pb in PCEE-10 increased from about 24.6 ng·kg^{-1} to 57.4 ng·kg^{-1}; and, in PCEE-100, from 182 ng·kg^{-1} to 451 ng·kg^{-1}.

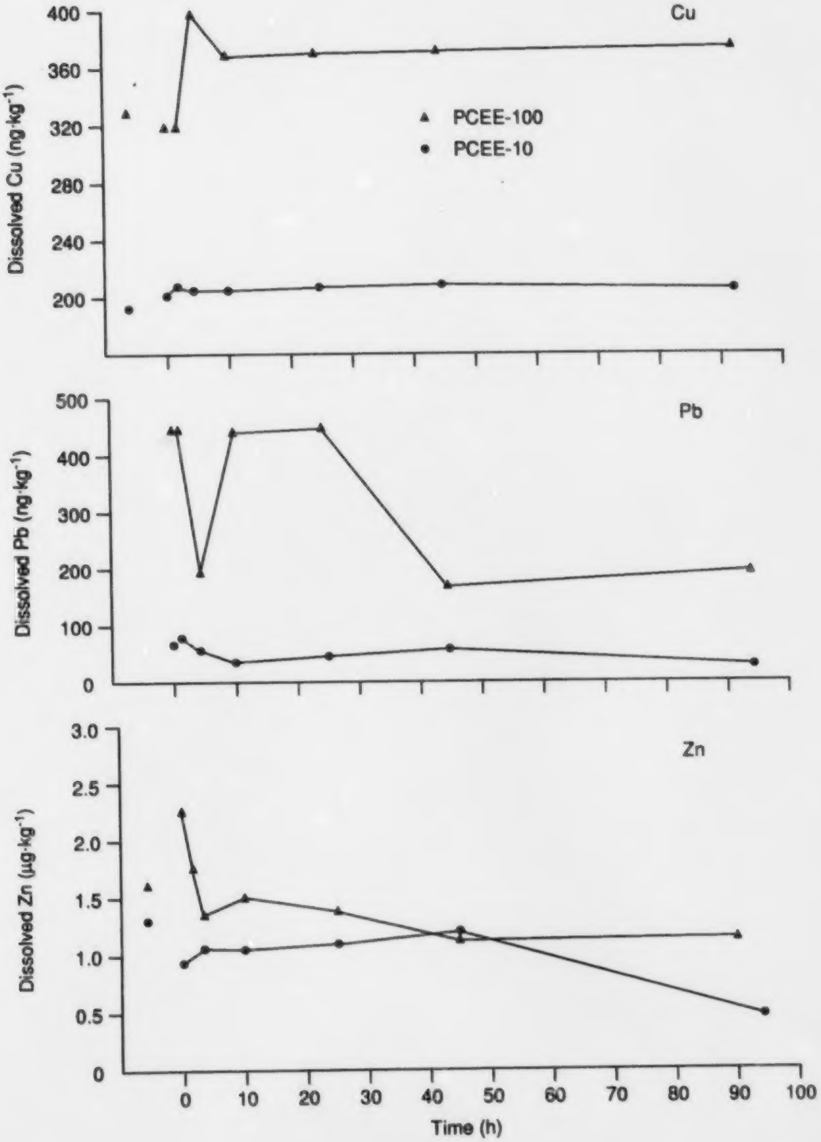

Fig. 5(a). Variations in the concentrations of dissolved trace metals in PCEE-10 and PCEE-100.

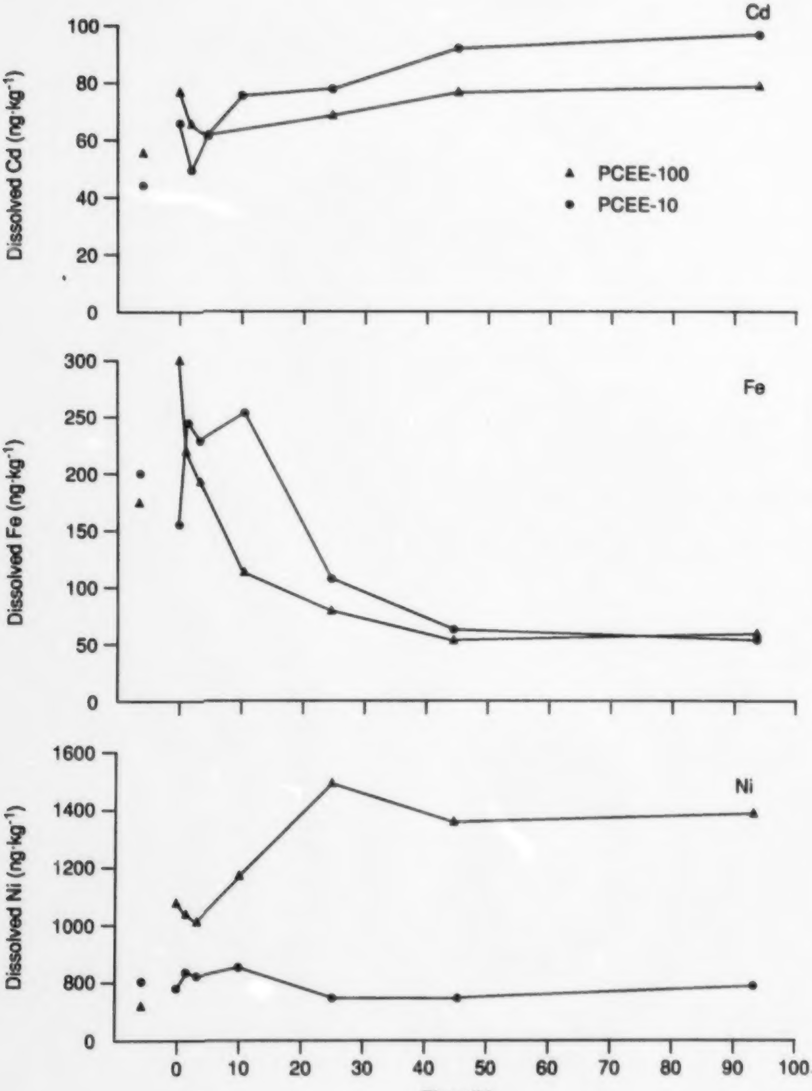

Fig. 5(b). Variations in the concentrations of dissolved trace metals in PCEE-10 and PCEE-100.

The concentrations then showed decreasing trends in general. At the end of the experiment, the concentration of dissolved Pb in PCEE-10 was even lower than the background level, whereas the concentration in PCEE-100 had returned to the background level. In both tanks, the concentrations of particulate Pb also showed an initial increase and then decreased gradually. At the end of the experiment, the

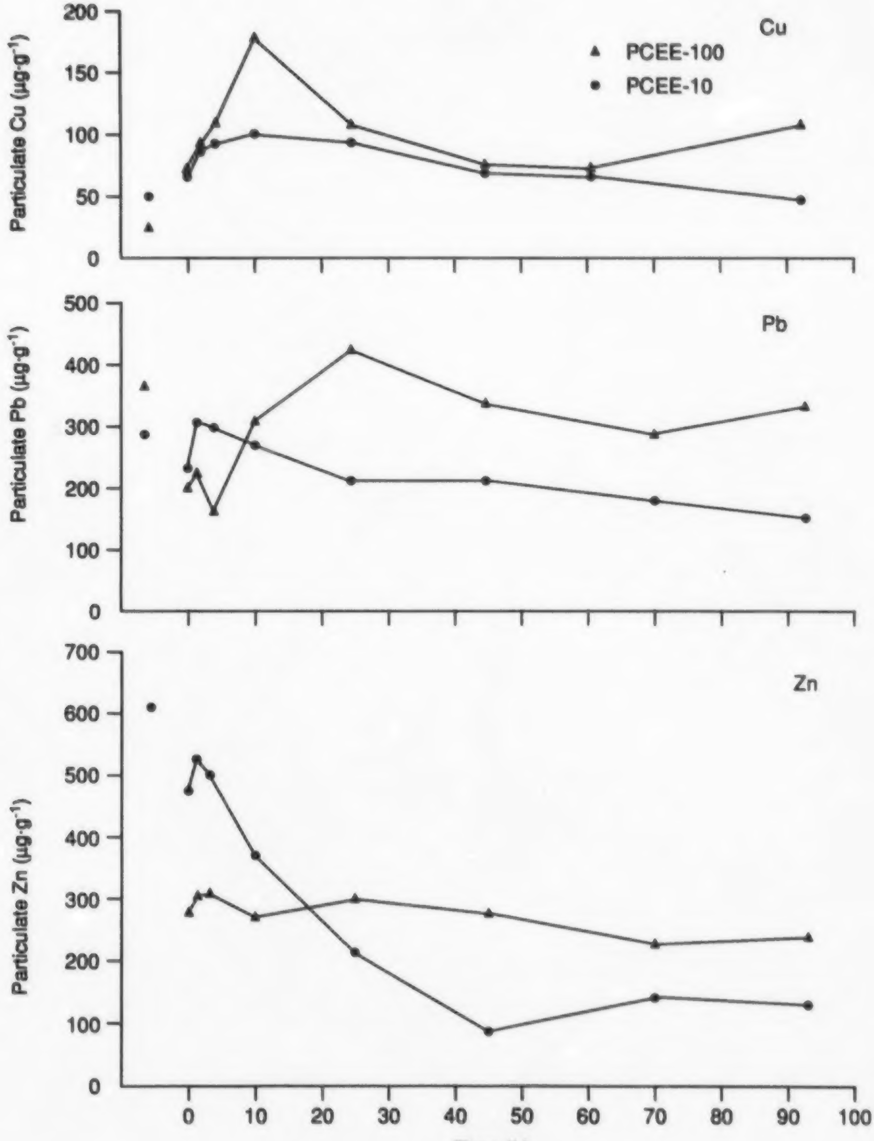

Fig. 6 (a). Variations in the concentrations of dissolved trace metals in PCEE-10 and PCEE-100.

concentration of particulate Pb in PCEE-10 was lower than the background level, whereas that in PCEE-100 was higher.

The behaviour of Zn in PCEE-10 differed from that in PCEE-100. As mine tail-

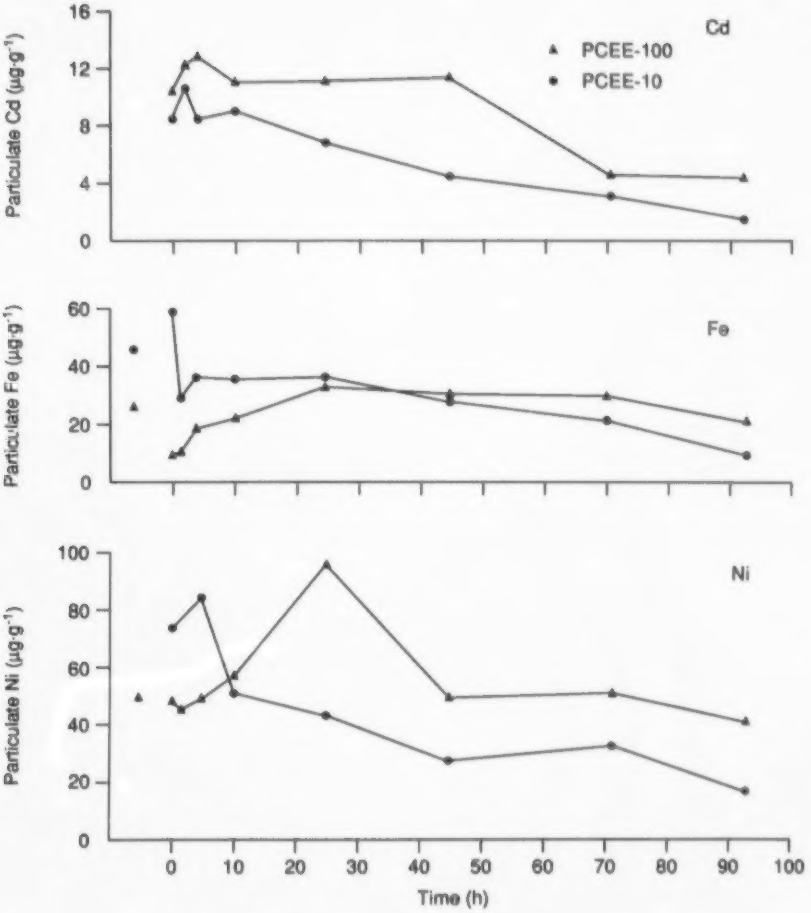

Fig. 6 (b). Variations in the concentrations of dissolved trace metals in PCEE-10 and PCEE-100.

ings were added to PCEE-10, the concentration of dissolved Zn decreased immediately to 26% below the background level and then remained at about the same level for the next 25 h, averaging 1.06 µg·kg^{-1} (sd = 8%). There was another significant decrease, 20 h later. Ninety-three hours after treatment, the concentration of dissolved Zn was only 0.49 µg·kg^{-1}, which was lower than the value before the addition of mine tailings. In PCEE-100, there was a 36% increase in the concentration of dissolved Zn right after treatment. During the experiment, the amount of particulate Zn in PCEE-10 remained fairly stable, whereas the amount in PCEE-100 increased with time.

After the addition of mine tailings, the concentration of dissolved Cd in PCEE-10 increased by 42%, from 46.7 ng·kg^{-1} to 66.1 ng·kg^{-1}, and then increased gradually to two times the background level by the end of the experiment. For PCEE-100, the concentration of dissolved Cd initially increased from 56.3 ng·kg^{-1} to 77.3 ng·kg^{-1}, and then remained at a stable level of 72.9 ng·kg^{-1} (sd = 10%; $n = 7$). Concentrations of particulate Cd in both PCEE-10 and PCEE-100 were quite similar; there were no large changes before the plankton blooms, but decreases occurred after the blooms.

The concentration of dissolved Fe in PCEE-10 increased 1.5 h after the addition of mine tailings, but then began decreasing after 10.5 h. At the end of the experiment, the concentration was only 54 ng·kg^{-1}, much less than the background level of 201 ng·kg^{-1}. A similar trend was observed in PCEE-100. The initial increase of 68% in the concentration of dissolved Fe was followed by a gradual decrease to a value of 61 ng·kg^{-1} by the end of the experiment. This value was also lower than the background level. For particulate Fe, concentrations decreased with time and final concentrations were lower than background levels.

Discussion

The concentration of nitrate is known to correlate closely with primary productivity and chlorophyll concentration (Takahashi et al. 1982). As primary productivity increases, a rapid loss of nitrate from the water body occurs (Parsons et al. 1984). This observation allows us to project the biological activities in an enclosed ecosystem from relationships between nitrate concentration and primary productivity, chlorophyll content, or the concentration of POC. Results from the present experiment demonstrated that variations in nutrient concentrations (nitrate, phosphate, and silicate), primary productivity, and chlorophyll content were very similar. In PCEE-10, during the first 25 h, nutrient concentrations showed no significant changes, and primary productivity was less than 2.6 mg C·m^{-3}·h^{-1}. Between 45 and 95 h, the concentration of nitrate decreased substantially, but primary productivity and amounts of chlorophyll and POC increased rapidly to their respective peak values. These phenomena were also observed in PCEE-100 but the transition point of the trends occurred 73 h after the addition of mine tailings.

At the beginning of the experiment, the concentration of phosphate increased from 1.56 μM to 2.66 μM. This increase probably resulted from dissolution of phosphate in the mine tailings.

The two treatment concentrations used in the present experiment, i.e., 10 and 100 ppm, simulated disposal concentrations of tailings from the Alice Arm mine into the ocean. Compared with the control (PCEE-C), the amount of mine tailings in PCEE-10 did not alter its primary productivity and chlorophyll content. However, the tailings seemed to affect biological activity in PCEE-100 by lowering primary productivity and the chlorophyll concentration and delaying the occurrence of the bloom. In PCEE-100 after 96 h, primary productivity (11.3 mg C·m^{-3}·h^{-1}) and chlorophyll concentration (2.87 mg·m^{-}3) were only 14 and 15%, respectively, of the peak values observed in PCEE-10.

The experimental observations can be divided into two stages according to the concentration of suspended particles and the activity of organisms. The first stage was a physicochemical process including the settling of large particles. During this stage, biological activity was low; thus the behaviour of trace metals depended mostly on interactions between the mine tailings and the seawater. Wong et al. (1986) indicated that 24 h after the addition of mine tailings, there were only 2–10 µ*M* of particulates in the water body. In the present study, variations in the concentrations of suspended particles were also very small (Fig. 4). The second stage included both physicochemical and biological processes. During this stage, most of the settling materials were organic detritus or resulted from the coagulation of fine particles.

In PCEE-10, the time separating these two stages was about 25 h after the addition of mine tailings; in PCEE-100, separation between the two phases occurred at about 73 h. Changes in species composition of the ecosystems, which are probably essential to understand the effects of mine tailings on the environment, were not observed. For example, the change in silicate concentration in PCEE-100 was more conspicuous than changes in the concentrations of nitrate and phosphate. This might reflect changing responses of different plankton species to mine tailings. Although the volume of seawater in PCEE-C was much less than in PCEE-10 and PCEE-100, many characteristics of the biological activities in PCEE-C and PCEE-100 were similar. However, the degree of similarity with respect to species composition in these two tanks was not known.

After the addition of mine tailings, the rapid increase in dissolved and particulate Fe (Figs 5e and 6e) was probably caused by the release of soluble Fe compounds in the mine tailings or by the transformation of insoluble Fe compounds into soluble Fe compounds. These releases were also noticed by Rohatzi and Chen (1975) in their study involving mixing polluted water with seawater. After these initial processes, concentrations of dissolved and particulate Fe decreased with time, probably due to hydrolysis of dissolved Fe or coagulation of colloidal Fe. Aston and Chester (1973) indicated that suspended particles could enhance precipitation of Fe. The effect of fine particulate matter was to increase both the settling rate and the settling mass of Fe in seawater. During the second stage, concentrations of dissolved and particulate Fe continued to decrease but exhibited no direct cause-and-effect relationship. Similarly, there were no close relationships between biological activity and the change in concentration of either dissolved Fe or particulate Fe. Other studies have also shown that scavenging by particulates is important in the transfer of Fe and Mn in the ocean (e.g., Singh and Subramanian 1984).

The concentration of total Cu (including dissolved and particulate Cu) increased significantly after the addition of mine tailings. In PCEE-10, the increase was 3.6 times; whereas in PCEE-100, it was 19 times. Thereafter, because of the settling of suspended particles, the amount of total Cu decreased, but the amount of dissolved Cu remained almost the same. Topping and Windom (1977) noticed that Cu is removed from seawater by adsorbing onto bodies of plankton and organic detritus. With respect to the present study, we speculate that, during the second stage of the experiment, two processes were in operation: continuous release of Cu from the mine tailings as well as complexing of dissolved Cu with inorganic or organic compounds and their subsequent adsorption onto suspended particles (Bourg 1982; Singh and Subramanian 1984). Copper was then removed from the water with the settling particles. Because the rates of these two processes were basically the same, the concentration of dissolved Cu also remained relatively unchanged.

Rohatzi and Chen (1975) suggested that Ni can be released from polluted water to seawater. In the present experiment, dissolved Ni increased initially, then decreased. During the first stage of the experiment, Ni was released from the tailings to the seawater. Thereafter, through various chemical reactions, such as adsorption of hydrous oxides, complexing and coprecipitation, metal was removed from the water body together with the settling particles. During the second stage, the concentration of dissolved Ni remained relatively stable, probably because of the equilibrium established between the rate of Ni release from the tailings and the transfer rate of Ni from other hydrous oxides and organic detritus. In PCEE-10, the concentration of particulate Ni decreased with the increase in biomass; whereas in PCEE-100, the concentration remained relatively stable because of the suppression of biotic activities.

As mine tailings were dispersed in seawater, the rate of Cd released from the tailings was higher than the transfer rate of Cd into the water. Early controlled ecosystem pollution experiment (CEPEX) studies supported the observation that the biological transfer of Cd was a slow process. Results from the present study also indicated that the concentration of dissolved Cd was still increasing at a much slower rate during the second stage of the experiment. Because the activity of organisms had little effect on the transfer of Cd, variations in the concentrations of dissolved Cd in PCEE-10 and PCEE-100 were basically the same.

The variations in dissolved Pb revealed in this experiment were also observed in a laboratory study (Hoff et al. 1982) and in an enclosure experiment (Wong et al. 1983). The decrease in dissolved Pb occurred mainly during the first stage suggesting that the transfer of Pb is a chemical process. Wong et al. (1983) also observed that Pb is not transferred by binding with settling organic matter. Although the concentration of dissolved Pb increased right after the addition of tailings, it returned to the background level by the end of the experiment, suggesting that the settling of suspended tailings particles was an important factor in the transfer of dissolved and particulate Pb.

Many studies have confirmed that Zn can be adsorbed onto hydrous ferric oxide or organic detritus. Zinc is also a micronutrient that can exist in a complex with other organic compounds. Thus, in the present study, Zn was probably transferred by physicochemical processes during the first stage of the experiment. During the second stage, Zn settled and was removed from the water body by complexing with organic compounds or by becoming adsorbed onto organic detritus. However, in PCEE-10, concentrations of Zn were reduced by 71 and 12% during the first and second stages respectively; whereas in PCEE-100, the reductions were 90 and 3% respectively. These results strongly suggest that Zn was transferred by some physicochemical processes.

Aston, S.R., Chester, R. 1973. The influence of suspended particles on the precipitation of iron in natural waters. Estuarine and Coastal Marine Science, 1, 225–231.

Bourg, C.M. 1982. Role of fresh water/seawater mixing on trace metal adsorption phenomena. *In* Wong, C.S., ed., Trace metal in seawater. Proceedings of a NATO Advanced Research Institute Conference, 30 March–3 April 1981, Erice, Italy. Plenum Press, New York, NY, USA.

Danielsson, L-G., Magnusson, B., Westerlund, S. 1978. An improved metal extraction procedure for the determination of trace metals in sea water by atomic absorption spectrometry with electrothermal atomization. Analytica Chimica Acta, 98, 47–57.

Eggimann, S.W., Betzer, R. R. 1976. Decomposition and analysis of refractory oceanic suspended materials. Analytical Chemistry, 48, 886–890.

Hoff, J.T., Thompson, J.A., Wong, C.S. 1982. Heavy metal release from mine tailings into sea water: a laboratory study. Marine Pollution Bulletin, 13, 283–286.

Parsons, T.R., Harrison, P.J., Acreman, J.C., Dovey, H.M., Thompson, P.A., Lalli, C.M., Lee, K., Li, G., Chen X. 1984. An experimental marine ecosystem response to crude oil and Corexit 9527 — Part 2: Biological effects. Marine Environmental Research, 13, 265–275.

Rohatzi, N., Chen, K.Y. 1975. Transport of trace metal by suspended particulates on mixing with sea water. Journal of the Water Pollution Control Federation, 47, 2 299.

Singh, S.K., Subramanian, V. 1984. Hydrous Fe and Mn oxides — Scavengers of heavy metals in the aquatic environment. CRC Critical Reviews in Environmental Control, 14, 33–90.

Strickland, J.D.H., Parsons, T.R. 1972. A practical handbook of sea water analysis. Bulletin of the Fisheries Research Board of Canada, 167, 310.

Takahashi, M., Koike, I., Iseki, K., Bienfang, P.K., Hattori, A. 1982. Phytoplankton species responses to nutrient change in experiment enclosures and coastal water. *In* Grice, G.D., Reeve, M., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 333–340.

Topping, G., Windom, H.L. 1977. Biological transport of copper at Loch Ewe and Saanich Inlet: controlled ecosystem pollution experiment. Bulletin of Marine Science, 27, 135–141.

Waldichuk, M. 1978. Disposal of mine wastes into the sea. Marine Pollution Bulletin, 9, 141–143.

Waldichuk, M., Buchanan, R.J. 1980. Significance of environmental changes due to mine waste disposal into Rupert Inlet: Federal–Provincial (British Columbia) review of the mine waste disposal problem in Rupert Inlet, British Columbia. Fisheries and Oceans Canada, West Vancouver, BC, Canada. 56 pp.

Wong, C.S., Kremling, K., Riley, J.P., Johnson, W.K., Stukas, V., Berrang, P.G., Erickson, P., Thomas, D., Peterson, H., Imber, B. 1983. An intercomparison of sampling devices and analytical techniques using seawater from a CEPEX enclosure. *In* Wong, C.S., ed., Trace metals in seawater. Proceedings of a NATO Advanced Research Institute Conference, 30 March–3 April 1981, Erice, Italy. Plenum Press, New York, NY, USA. 175–193.

Wong, C.S., Whitney, F.A., Johnson, W.K., Lu, X., Wu J., Wu S., Li J.. 1986. The effect of mine tailings on levels of heavy metals in seawater. Acta Oceanologica Sinica, 5, 401-415.

Effects of Liaohe Crude Oil and Dispersant on Marine Phytoplankton in an Enclosed Ecosystem

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An enclosed ecosystem experiment was carried out in eight plastic bags, each containing 2.5 m^3 *of seawater, over a period of 23 d to examine the effects of Liaohe crude oil and dispersant on the growth of marine phytoplankton from Xiaopingdao Bay, Yellow Sea. Results showed that Liaohe crude oil (at about 120 ppm) stimulated the growth of several small species of phytoplankton and caused an increase in chlorophyll a concentration. The dispersant alone (at 40–120 ppm) or mixed with crude oil was inhibitory, reducing the number of species and the chlorophyll a concentration. When phytoplankton were exposed to the oil and dispersant mixture, they recovered after about 11 d.*

With the development of the oil industry, oil-spill accidents frequently occur and oil pollution has become a concern. Many studies have been carried out on the effects of oil on marine organisms and marine environments. Previous results on the effects of oil on phytoplankton have varied because of the different experimental methods, conditions, and types of oils used (Canevari and Lindblom 1976; Elmgren and Frithsen 1982; Hartwick et al. 1982; Dahl et al. 1983; Grothers 1983; Østergaard et al. 1984). When dispersants are used to control oil spills, complicated problems may arise as a result of the dispersants themselves or their mixing with oils in different proportions, which may affect marine organisms. Therefore, it is necessary to conduct experiments under controlled conditions that simulate real situations.

The experiment reported in this paper was performed in an enclosed ecosystem under field conditions. The purpose of the study was to determine the response of a natural phytoplankton assemblage in the enclosed ecosystem to Liaohe crude oil at various concentrations and mixed with domestic Shuangxiang No. 1 dispersant in various proportions. This information should be useful for controlling agencies that use this dispersant during oil-spill cleanups.

Materials and methods

The experiment was conducted in Xiaopingdao Bay, 300–400 m offshore within a *Laminaria* seaweed-farming area in the Yellow Sea. The eight polyethylene bags used in this experiment had a diameter of 1 m, were 2.5 m long, and contained 2.5 m^3 of seawater.

The crude oil used in the experiment was obtained from the petroleum port of Dalian Harbour. Gas chromatographic analysis showed 64.4% n-alkanes and 35.6% aromatic and cyclic alkanes. Among the n-alkanes, 59.3% were nC9–nC18 and 40.7% were nC19-nC30. The dispersant used in the experiment was domestic Shuangxiang No. 1 obtained from the Second Organic Chemical Plant. Its boiling point was >75°C and its viscosity at 30°C was <10 cP (0.01 Pa·s). Its emulsifying efficiency (30-s mixing) was >60%. Its composition was 75% liquid wax (containing 1% aromatic compounds), 18% oleic acid ($C_{18}H_{34}O_2$), and 17% oxirane.

The eight plastic bags were filled with seawater on 3 September 1986. After they were filled, seawater samples were taken inside each bag and outside the bags to determine oil content, chlorophyll *a*, pH, dissolved oxygen, inorganic nitrogen (NO_3^-, NO_2^-, and NH_4^+), and numbers and species composition of phytoplankton. These measurements represented the initial values (day 0) of the experiment. After the initial samples were taken, oil and dispersant were added as indicated in Table 1: the amounts of oil and dispersant added in this experiment were relatively large. The purpose was to simulate a large oil spill and observe the short-term responses of phytoplankton to oil and also the consequence of using the dispersant directly without another treatment after an oil spill. Samples were taken on days 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 17, and 23.

Samples for oil and oxygen determinations were taken separately. All other samples were collected from the 0–2.5 m layer using a 2.5-m long tube. Samples were mixed and split for various determinations. Chlorophyll *a* was measured and calculated according to the method recommended by Unesco (1966). The oil concentration in the seawater was determined by the ultraviolet method according to SOA (1979). Other chemical analyses followed those methods outlined in SOA (1975, 1979).

A 500-mL subsample for phytoplankton counting was fixed with Lugol's

Table 1. Concentrations (mL per bag) of crude oil and dispersant added to the eight bags.

Bag	Crude oil	Dispersant
0	0	0
1	0	0
2	300	0
3	1 500	0
4	3 000	0
5	0	150
6	300	100
7	300	300
8	300	200

Note: Each bag contained 2 500 L of seawater.

solution and concentrated to 50 mL by gravity settling. From the 50 mL sample, 0.5 ml was taken and counted under a microscope at ×100 magnification. For the dominant species, at least 200 cells were counted. For some samples in which cell numbers and species varied greatly, they were counted twice.

As a result of the emulsion conditions of the seawater in bags 5–8 after adding the dispersant, some chemical measurements could not be made. Only qualitative and quantitative data for chlorophyll *a* and phytoplankton were obtained. Because of the shallow depth of the bags, the data we obtained can be considered as the average values for the enclosed water body in the bags.

Results and discussion

During the experiment, the natural seawater outside the bags experienced a temperature range of 19–21.5°C, and contained 0.1–0.6 μ*M* inorganic nitrogen, 34.5–70.4 $\mu g \cdot L^{-1}$ oil, 14 000–110 000 $cells \cdot L^{-1}$ total phytoplankton, and 0.08–1.9 $\mu g \cdot L^{-1}$ chlorophyll *a*.

Responses of phytoplankton to crude oil

After the addition of oil to each bag, oil concentrations reached their peak values 1 d later in bag 2 (0.87 $mg \cdot L^{-1}$), on day 2 in bag 3 (1.60 $mg \cdot L^{-1}$), and on day 3 in bag 4 (2.06 $mg \cdot L^{-1}$),and then declined (Fig. 1). These results indicated that the seawater's capacity for oil decreased as the concentration of oil added increased.

Effects of crude oil on phytoplankton biomass

The total number of phytoplankton in the control and the oil only experimental bags (bags 1–4) increased from day 1 (Fig. 2). The number in the control (bag 1) reached a peak (1.5×10^6 $cells \cdot L^{-1}$) on day 2, after which it declined rapidly. Total cell numbers in the three treated bags decreased much more slowly after reaching their peaks. Results from the present study indicated that the more crude oil added,

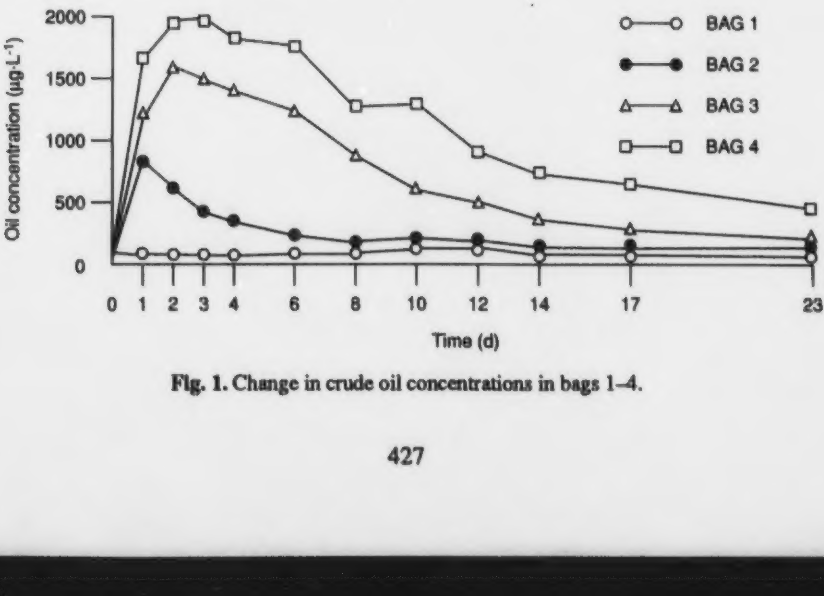


Fig. 1. Change in crude oil concentrations in bags 1–4.

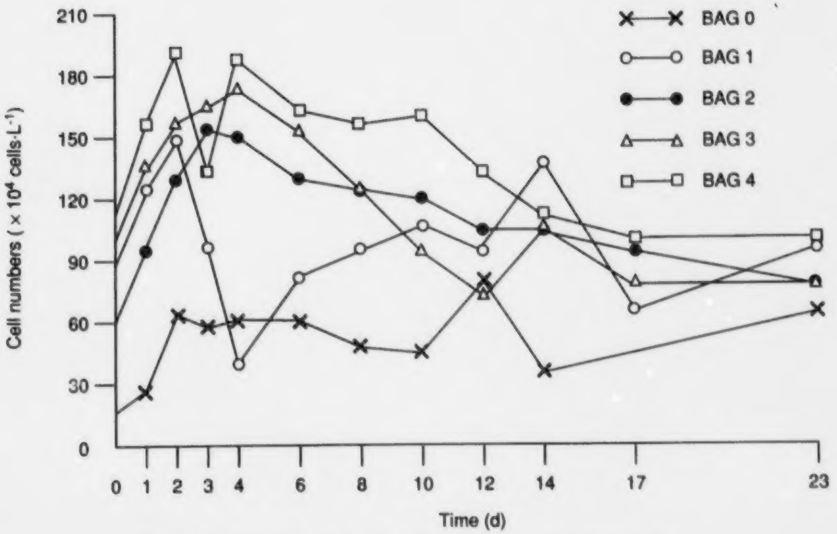


Fig. 2. Change in total phytoplankton cell numbers in bags 0–4.

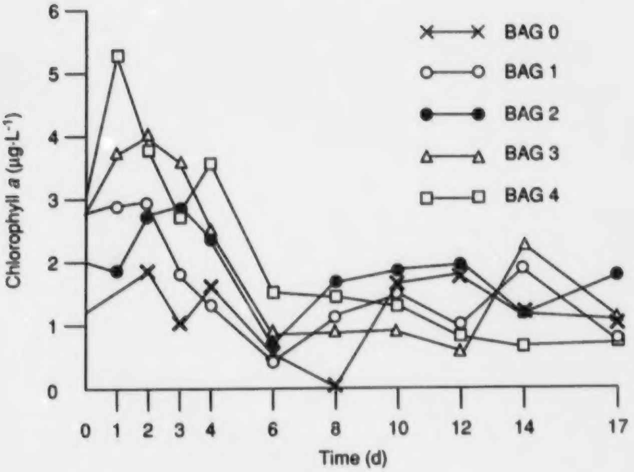


Fig. 3. Change in chlorophyll *a* concentrations in bags 1–4.

the higher the total phytoplankton cell numbers (Fig. 2). This suggests that the crude oil used in this experiment stimulated phytoplankton growth.

Chlorophyll *a* concentrations (Fig. 3) in each bag displayed a trend similar to that displayed for total cell numbers except that declines in chlorophyll *a* concentrations were more rapid. In this experiment, the major factor inhibiting increases in phytoplankton biomass was the consumption of nutrients. Analyses of nutrients showed that ammonium made up 70% of the total inorganic nitrogen over the whole experimental period. Because phytoplankton prefer using ammonium (McCarthy 1980), only changes in ammonium were analyzed.

Changes in ammonium concentrations in each bag exhibited similar trends (Fig. 4), with no apparent differences being observed among the bags. Toward the end of the experiment, ammonium concentrations increased in the bags, possibly because of the release of ammonium during decomposition of the bloom. An enclosed ecosystem experiment conducted in the Xiamen area of China demonstrated that phosphorus limited phytoplankton growth (Harrison et al., this volume).

Effects of crude oil on phytoplankton species succession

The change in phytoplankton species composition was complicated, and will be discussed in another paper. Here, a brief description of the general trend is presented.

The total number of species did not vary greatly among the bags. Therefore, the oil addition of oil did not affect species diversity. Figure 5 shows the proportion of dominant species relative to the total cell number in each bag and their succession. Seawater outside the bag (bag 0) was characterized by a typical fall phytoplankton assemblage of small diatoms, among which the most abundant species was *Skeletonema costatum* (68%). The next most abundant species were *Chaetoceros* spp and *Leptocylindrus danicus*, 10 and 20% respectively. Some dinoflagellates were present (about 10%); this group was composed of small species, including *Gonyaulax* spp, *Ceratium fusus*, and *Prococentrum micans*.

The control bag (bag 1) and the other, oil-treated bags had different successions of dominant species and also different succession times. As the concentration of oil increased, the period during which *Skeletonema costatum* was the dominant species became longer (Fig. 5). Similarly, *Chaetoceros* spp appeared later and lasted longer in the bags receiving larger additions of oil. The dominant period for

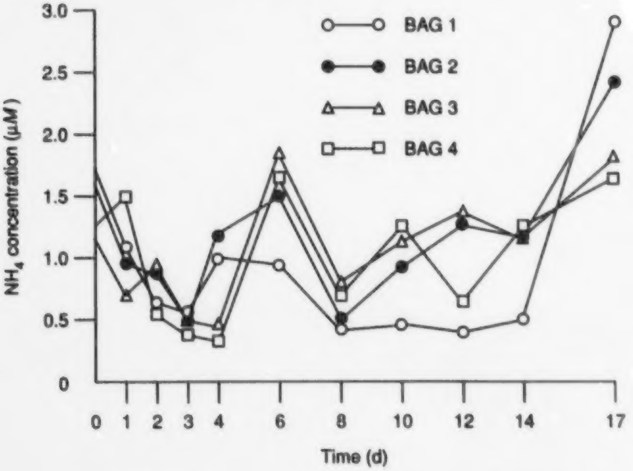
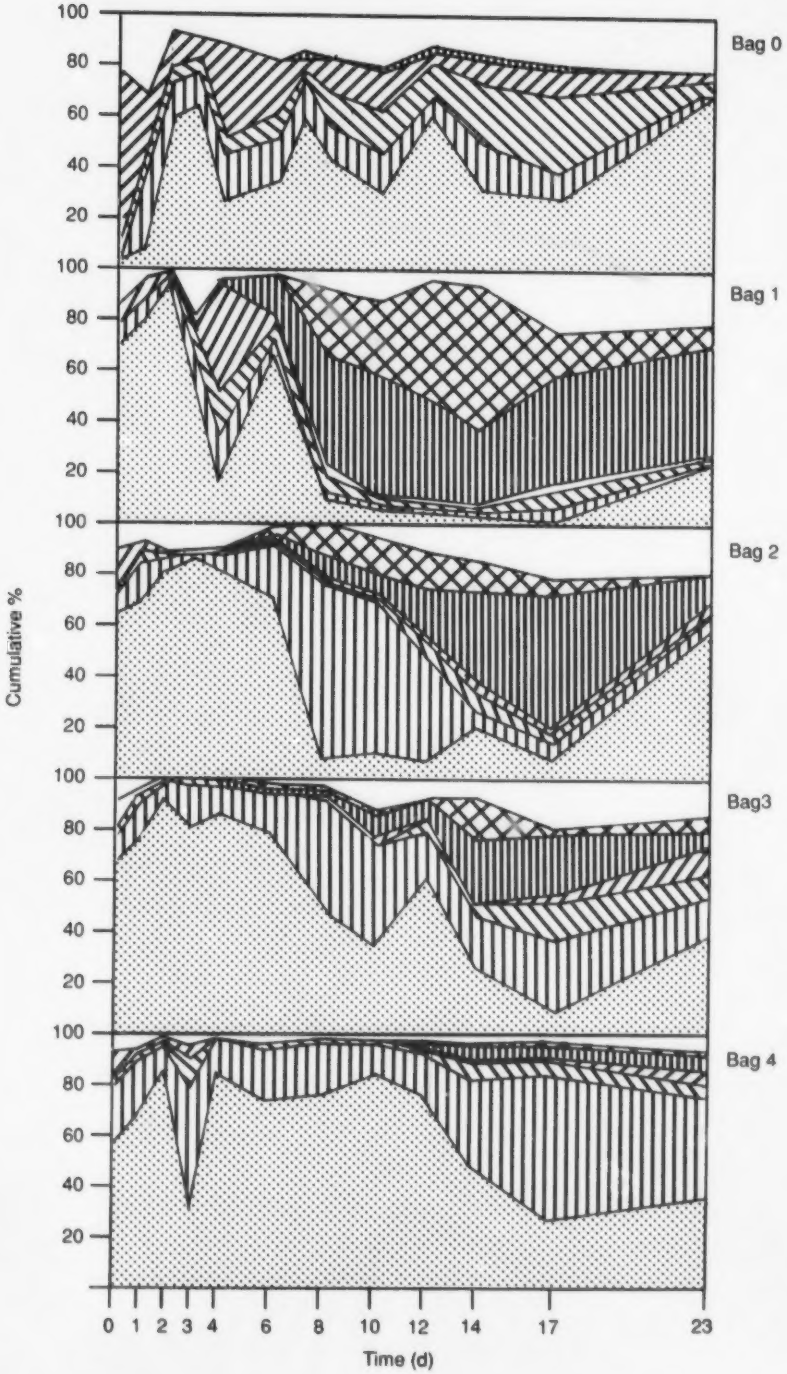


Fig. 4. Change in ammonium concentrations in bags 1–4.


Bag 0
Bag 1
Bag 2
Bag3
Bag 4
Cumulative %
100
80
60
40
20
100
80
60
40
20
100
80
60
40
20
100
80
60
40
20
100
80
60
40
20
0
1
2
3
4
6
8
10
12
14
17
23
Time (d)

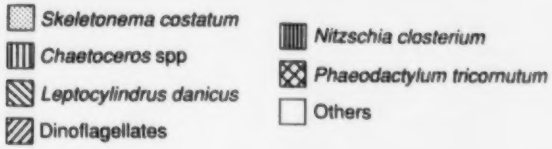


Fig. 5. Change in cumulative percentages of dominant phytoplankton species in bag 0 (sample from outside bags), bag 1 (control), bag 2 (300 mL of oil added), bag 3 (1 500 mL of oil added), and bag 4 (3 000 mL of oil added). The dominant species or groups were: *Skeletonema costatum*, *Chaetoceros* spp, *Leptocylindrus danicus*, dinoflagellates, *Nitzschia closterium*, and *Phaedactylum tricornutum*.

Phaeodactylum tricornutum and *Nitzschia closterium* was shortened and their dominance decreased and eventually they disappeared in bags receiving more oil.

In summary, it was found that this type of crude oil stimulated the growth of *Skeletonema costatum* and that its percentage increase roughly paralleled the increase in oil concentration in bags 1–4 (Fig. 5). However, the crude oil inhibited the growth of *Phaeodactylum tricornutum* and *Nitzschia closterium*. *Chaetoceros* spp were least responsive to the crude oil, their numbers increasing in proportion to total cell numbers in each bag.

Response of phytoplankton to the dispersant and oil–dispersant mixture

Effects on phytoplankton biomass

Addition of a high concentration of dispersant alone and its mixture with crude oil depressed phytoplankton growth during the first few days (Fig. 6). After day 6, the phytoplankton recovered to some extent and became stable after 14 d, reaching a biomass similar to that in the oil-only bags (bags 1–4).

Cell numbers in bag 8 were different from those in the other bags during the recovery process after day 4; as well, the chlorophyll *a* concentration in bag 8 was higher. This was mainly due to the growth of *Skeletonema costatum*. Growth of this species in the other bags was suppressed, but the reason for this was not clear.

These results did not agree with those of similar experiments conducted by Harrison et al. (1986) in Canada, perhaps due to the higher oil concentrations added and differences in the type of oil and dispersant used. The concentrations of crude oil and dispersant added in the present experiment were 120 ppm, and 40–120 ppm respectively; whereas only 3 ppm crude oil and 0.3 ppm dispersant were added in the Canadian experiment. Many small particles were observed sticking to the surface of the phytoplankton cell. The addition of dispersant resulted in a white emulsion and turbid conditions in the seawater, whereas the addition of oil and the dispersant–oil mixture led to a yellow–brown turbid emulsion. After 10 d, the seawater in these bags became clear and the cell surface of the phytoplankton had no particles stuck to it. Therefore, whether the effects of the dispersant at high concentrations on phytoplankton were chemical (toxicity) or physical needs to be investigated further. Under the above conditions, particles sticking to cell surfaces must affect the normal physiological activities of phytoplankton. Based upon the result

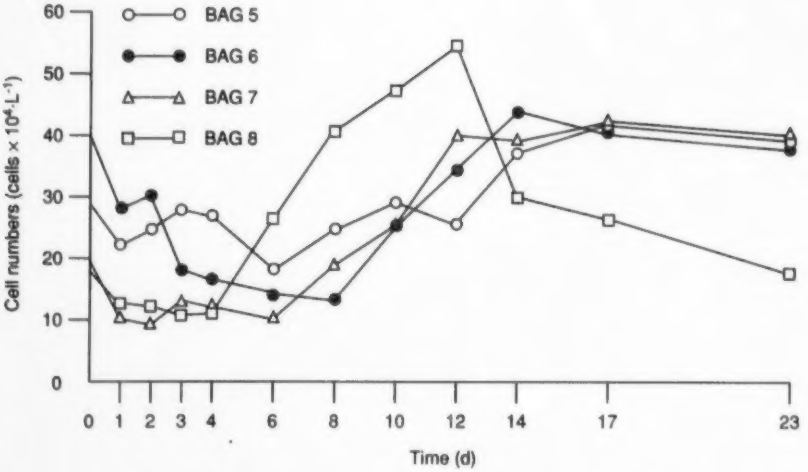


Fig. 6. Change in total phytoplankton cell numbers in bags 5–8.

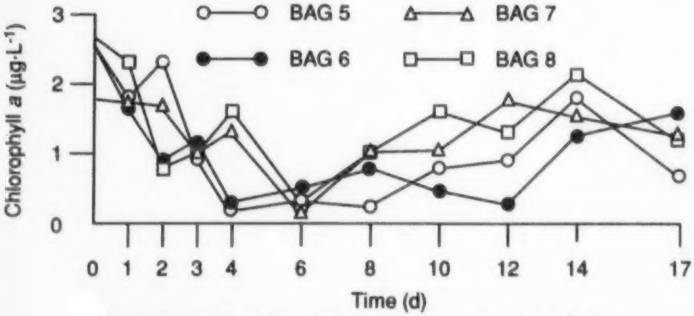


Fig. 7. Change in chlorophyll *a* concentrations in bags 5–8.

of some indoor experiments and other unpublished data, this dispersant is not too toxic to zooplankton and higher animals.

Effects on phytoplankton species composition

High concentrations of the dispersant and its mixture with oil depressed phytoplankton and greatly reduced total cell numbers initially (Fig. 6). After 10 d, cell numbers in the treated bags recovered to similar numbers as was observed in the control. This recovery has been observed in previous oil experiments (Dahl et al. 1983; Elmgren and Frithsen 1982; Harrison et al. 1986). There was some reduction in the number of species (i.e., a decrease in diversity), but this reduction was probably not significant. Phytoplankton species composition in bags 5–8 was a stable assemblage (Fig. 8). What differed from the natural assemblage outside the bags was that its most abundant species, *Skeletonema costatum*, was replaced with *Chaetoceros* spp (about 60%). *Skeletonema costatum* became the second most dominant species (30–40%), with the proportion of this species decreasing as the dispersant concentration increased. Therefore, the major factor affecting phytoplankton

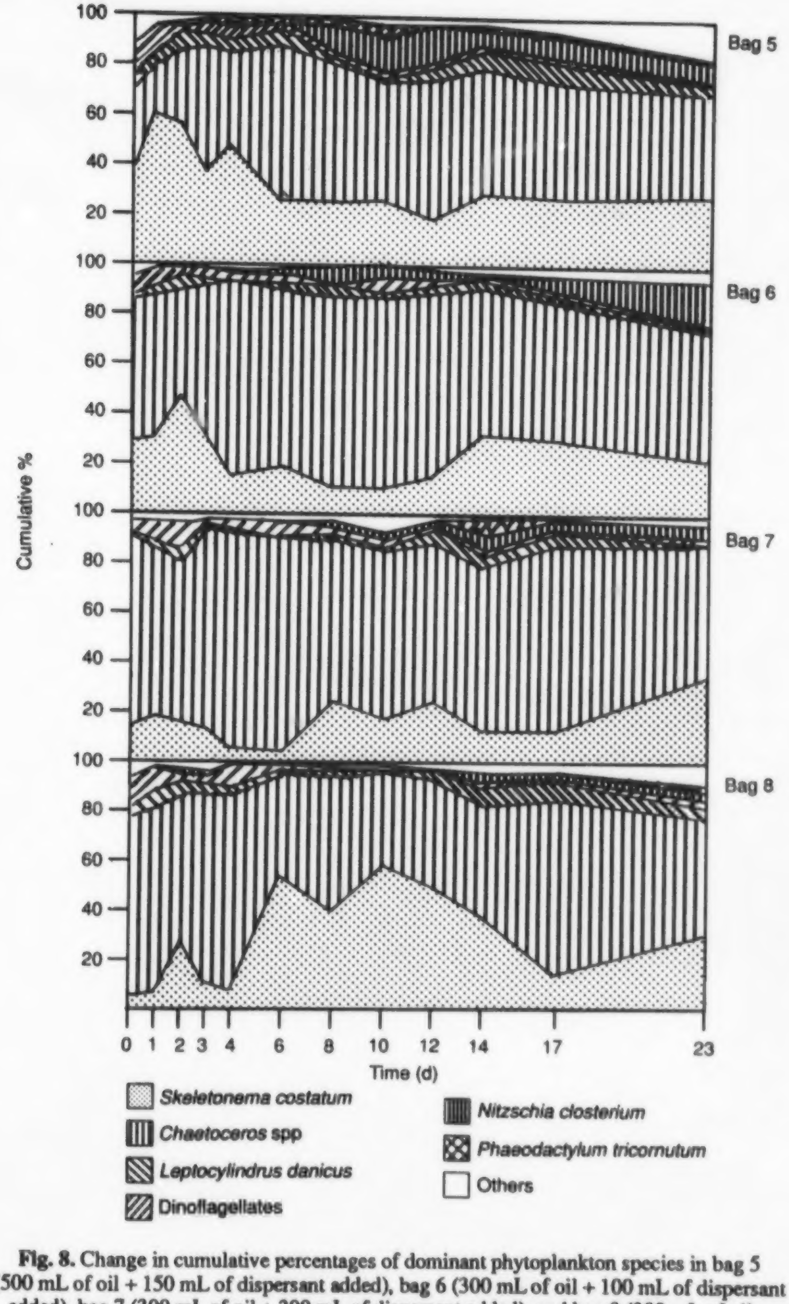


Fig. 8. Change in cumulative percentages of dominant phytoplankton species in bag 5 (500 mL of oil + 150 mL of dispersant added), bag 6 (300 mL of oil + 100 mL of dispersant added), bag 7 (300 mL of oil + 300 mL of dispersant added), and bag 8 (300 mL of oil + 200 mL of dispersant added).

growth was the dispersant, the effects of crude oil being relatively small. Also, *Skeletonema costatum* was more sensitive to the dispersant than *Chaetoceros* spp.

Summary

For the range of concentrations used in this experiment, Liaohe oil stimulated phytoplankton growth due to the fast increase in opportunistic species. It also affected phytoplankton species succession. Generally, the crude oil stimulated the growth of *Skeletonema costatum*, but depressed the growth of pennate diatoms.

The high concentration of dispersant (40–120 ppm) and its mixture with oil inhibited phytoplankton growth, but the degree to which species were affected was different. The natural assemblage of phytoplankton had a strong ability to recover. With a mixture of dispersant and oil, the assemblage recovered in 10–15 d.

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Canevari, G.P., Lindblom, G.P. 1976. Some dissenting remarks on deleterious effects of Corexit 9527 on fertilization and development. Marine Pollution Bulletin, 7, 127–128.

Dahl, E., Lake, M., Tjessem, K., Eberlein, K., Bohle, B. 1983. Effects of Ekofisk crude oil on an enclosed planktonic ecosystem. Marine Ecology Progress Series, 14, 81-91.

Elmgren, R., Frithsen, J.B. 1982. The use of experimental ecosystems for evaluating the environmental impact of pollutants: a comparison of an oil spill in the Baltic Sea and two long-term, low-level oil addition experiments in microcosms. *In* Grice, G.D., Reeve, M.R., ed., Marine mesocosms: biological and chemical research in experimental ecosystems. Springer-Verlag, New York, NY, USA. 153–165.

Grothers, J.H. 1983. Field experiments on the effects of crude oil and dispersant on common animals and plants of rocky seashores. Marine Environmental Research, 8, 219–239.

Harrison, P.J., Cochlan, W.P., Acreman, J.C., Parsons, T.R., Thompson, P.A., Dovey, H.M. 1986. The effects of crude oil and Corexit 9527 on marine phytoplankton in an experimental enclosure. Marine Environmental Research, 18, 93–109.

Hartwick, E.B., Wu, R.S.S., Parker, D.B. 1982. Effects of a crude oil and crude oil dispersant (Corexit 9527) on populations of the littleneck clam (*Protothaca staminea*). Marine Environmental Research, 6, 291–306.

McCarthy, J.J. 1980. Nitrogen. *In* Morris, I., ed., The physiological ecology of phytoplankton. University of California Press, Los Angeles, CA, USA. 191–233.

Østergaard, K., Eide, I., Jensen, A. 1984. Exposure of phytoplankton to Ekofisk crude oil. Marine Environmental Research, 11, 183–200.

Tilseth, S., Solberg, T.S., Westrheim, K. 1984. Sublethal effects on the water-soluble fraction of Ekofisk crude oil on the early larval stage of cod (*Gadus morhua*). Marine Environmental Research, 11, 1–16.

Unesco (United Nations Educational, Scientific and Cultural Organization). 1966. Determinations of photosynthetic pigments in seawater. Unesco, Paris, France. Monographs on Oceanographic Methods 1, 69 pp.

Winters, W., O'Donnell, R., Batterton, J.C, Van Baalen, C. 1976. Water soluble components of four fuel oils: chemical characterization and effects on growth of microalgae. Marine Biology, 36, 269–276.

SOA (State Oceanic Administration). 1975. The standards for marine investigations. SOA, Xiamen, People's Republic of China.

______ 1979. The temporary standards for marine pollution investigations. SOA, Xiamen, People's Republic of China.

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